FORMULATION AND *IN VITRO* EVALUATION OF LAMIVUDINE AND ZIDOVUDINE CONTROLLED RELEASE BILAYER MATRIX TABLETS

Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY, Chennai – 32

In partial fulfillment for the award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

Reg. No: 26103012

Under the guidance of

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J.K.K. NATTRAJA COLLEGE OF PHARMACY

KOMARAPALAYAM – 638 183,

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MAY – 2012

Certificates

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION AND *IN VITRO* EVALUATION OF LAMIVUDINE AND ZIDOVUDINE CONTROLLED RELEASE BILAYER MATRIX TABLETS",** submitted by the student bearing **Reg. No. 26103012** to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACEUTICS** was evaluated by us during the examination held on…….

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"FORMULATION AND *IN VITRO* EVALUATION OF LAMIVUDINE AND ZIDOVUDINE CONTROLLED RELEASE BILAYER MATRIX TABLETS",** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACEUTICS**, is a bonafide work carried out by **Mr. SACHIN VITTHAL GOWARDIPE, [Reg.No:26103012],** during the academic year 2011-2012, under the guidance and direct supervision of **Mr. K. JAGANATHAN, M.Pharm.,** Lecturer, Department of Pharmaceutics**,** J.K.K. Nattraja College of Pharmacy, Komarapalayam.

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DECLARATION

I hereby declare that the dissertation entitled **"FORMULATION AND *IN VITRO* EVALUATION OF LAMIVUDINE AND ZIDOVUDINE CONTROLLED RELEASE BILAYER MATRIX TABLETS",** was carried out by me, under the guidance of **Mr. K. JAGANATHAN, M.Pharm.,** Lecturer, for submission to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACEUTICS**, The work is original and has not been submitted in part (or) any degree of this (or) any other university. The information furnished in this dissertation is genuine to best of my knowledge and belief.

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Mr. SACHIN VITTHAL GOWARDIPE

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Dedicated to

My Beloved Parents,

Lovely Friends

& Guide

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Introduction

1. INTRODUCTION

1. HIV AND AIDS[33]

In June 2006, a cumulative total of approximately 80 000 cases of HIV infection had been reported in the UK and 21 000 of these individuals had acquired immunodeficiency syndrome (AIDS), of whom 80% had died. Approximately 7500 new cases of HIV were reported in the UK in 2005. The most recent World Health Organization (WHO) report estimated that 38.6 million adults and 2.3 million children world-wide were living with HIV at the end of 2005. Globally, heterosexual transmission accounts for 85% of HIV infections. During 2005, an estimated 4.1 million became newly infected with HIV and an estimated 3 million people died from AIDS. World-wide, the HIV incidence rate is believed to have peaked in the late 1990s and to have stabilized subsequently, notwithstanding an increasing incidence in South-East Asia and China.

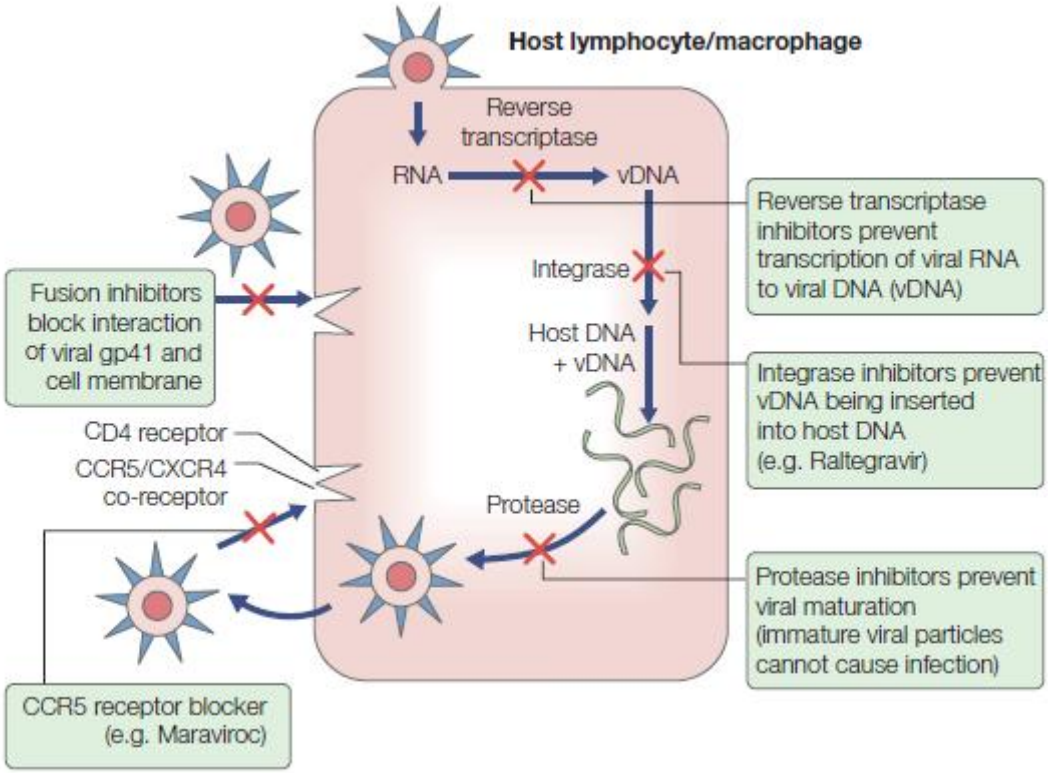


Fig. No. 1: Sites of action of anti-HIV drugs.

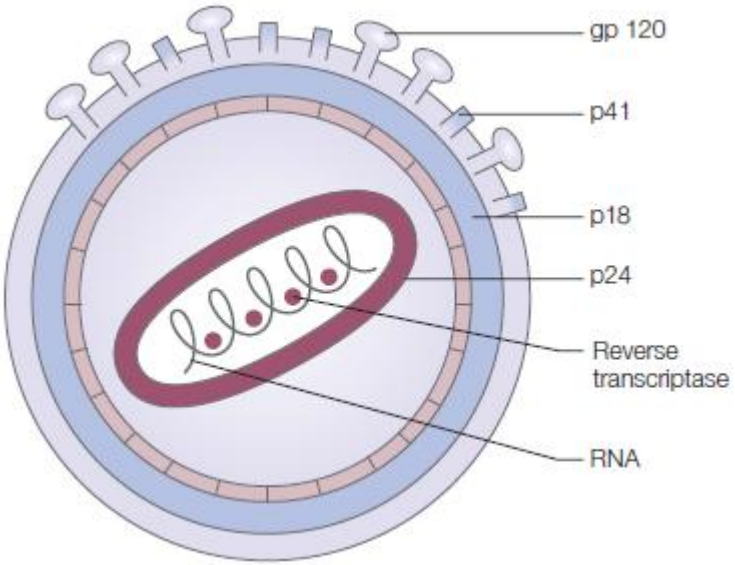


Fig. No. 2: HIV structure consisting of membrane glycoprotein gp120 and peptide protein p41 plus an outer membrane of p18 and a nuclear membrane of p24 protein containing viral RNA and the HIV-1 reverse transcriptase and integrase.

Above inoculation of a naive host with biological fluid (e.g. blood, blood products or sexual secretions) containing HIV-1, the virus adheres to cells, e.g. lymphocytes, macrophages and dendritic cells in the blood, lymphoid organs or central nervous system, expressing the CD4 receptor and chemokine coreceptors (e.g the CXC chemokine receptor 4 (CXCR4) and the chemokine receptor 5 (CCR5)). During entry, gp120 attaches to the cell membrane by binding to the CD4 receptor. Subsequent interactions between virus and chemokine co-receptors (e.g. CXCR4 and CCR5) trigger irreversible conformational changes. The fusion event takes place within minutes by pore formation and releases the viral core into the cell cytoplasm. The virus then disassembles and the viral reverse transcriptase produces complementary DNA (cDNA) coded by viral RNA. This viral DNA is then integrated into the host genome by the HIV-1 integrase enzyme. Viral cDNA is then transcribed by the host, producing messenger RNA (mRNA) which is translated into viral peptides. These peptides are then cleaved by HIV protease to form the structural viral proteins that, together with viral RNA, assemble to form new infectious HIV virions. These exit the cell by endosomal budding. Figure 8. illustrates the HIV-1 life cycle, together with current and potential therapeutic

targets. Newly formed HIV-1 virions infect previously uninfected CD4/CCR5-positive cells and subsequently impair the host immune response by killing or inhibiting CD4/CCR5-positive cells, thus rendering the host immunosuppressed and consequently at high risk of infections by commensal and opportunistic organisms. The diagnosis of HIV-1 infection is based on a combination of the enzyme-linked immunosorbent assay (ELISA) techniques that identify HIV-1 antibodies and Western blotting is then used to confirm the presence of HIV-1 structural nproteins in blood (see Figure No. 2). Massive viral replication n(3 _ 109 virions per day) occurs in the four to eight weeks immediately post HIV-1 infection. Viral replication falls in 8–12 weeks, and stabilizes within 6–12 months, initiating a latentmnperiod of good health which may last 5–12 years. During this latent period, the viral load falls from an initial peak and remains stable at a plateau of 102–106 HIV RNA copy number/mL of plasma. The HIV RNA copy number then rises before the development of AIDS. During the latent phase, there is a dynamic equilibrium of HIV replication, T-cell infection and destruction and new T-cell generation with a slow and inexorable decline in CD4_ cell numbers. Only after theCD4 lymphocyte cell count has fallen to 200–500/_L is the individual predisposed to opportunistic infections (e.g. pneumocystis, tuberculosis) or malignancies (e.g. Kaposi's sarcoma, lymphoma). It is these infections and malignancies that define the later stages of HIV-1 infection, known as AIDS.

GENERAL PRINCIPLES FOR TREATINGGENERAL PRINCIPLES FOR TREATING HIV-SEROPOSITIVE INDIVIDUALS:

The accepted standard for HIV treatment is that combination highly active antiretroviral therapy (HAART) should be administered before substantial immunodeficiency intervenes. The primary aim of treating patients with HIV infection is maximal suppression of HIV replication for as long as possible. This improves survival. HAART comprises two nucleoside analogues plus either a boosted protease inhibitor or a non-nucleoside reverse transcriptase inhibitor and reduces viral load to 500 copies of HIV RNA/mL in 80% of patients after 12 months treatment. Not all patients tolerate triple therapy due to toxicity, and alternate double therapy may be used. Current British HIV Association (BHIVA) recommendations

for initiating anti-HIV therapy are as follows. All HIV seropositive patients with symptoms (or AIDS-defining disease) should receive HAART. If the CD4 count is 350 cells per _L or if there is a rapid decline in CD4 count of _300 cells per _L over 12 months, such patients should be treated. Treatment may be deferred and the patient monitored if asymptomatic and CD4 counts are stable in the range 350–500 cells per _L. The recommended regimens for initial therapy are shown and are expected to reduce the HIV RNA copy number per mL of plasma by _0.5 log by week 8 of therapy, and ultimately to undetectable levels, and to maintain this state. The plasma HIV RNA copy number is the accepted gold standard for monitoring therapy and is inversely correlated with CD4 count and survival. HIV therapy guidelines are evolving rapidly, requiringspecialist care. Current principles emphasize combination therapy, regime convenience, tolerability and lifelong therapy. Anti- HIV therapy is a complex therapeutic arena, necessitating specialist supervision.

NUCLEOSIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITORS (NRTIs):

Of these agents, only zidovudine (ZDV) has been proved to reduce mortality in late-stage AIDS. It reduces the incidence of opportunistic infections and possibly also the rate of progression of HIV-1 infection to AIDS. Other members of the class include lamivudine (3-TC), stavudine (d4T), didanosine (ddI), emtricitabine (FTC) and abacavir (ABC). These drugs are used in combinations and are available as combined products, e.g. 3-TC/ZDV, ABC/3-TC, ZDV/tenofovir. They reduce HIV-1 viral replication as indicated by plasma HIV RNA load.

ZIDOVUDINE (AZIDOTHYMIDINE)

This was originally synthesized in 1964 in the hope that it would be useful in treating malignancies. These hopes were not fulfilled, but it was the first nucleoside analogue effective in treating HIV-1 infection.

Mechanism of action

The parent drug, ZDV, enters virally infected cells by diffusion and undergoes phosphorylation first to its monophosphate (ZDV-MP) then to the diphosphate (ZDV-DP), the rate-limiting step, and finally to the triphosphate (ZDV-TP). The intracellular *t*1/2 of ZDV-TP is two to three hours. ZDV-TP is a competitive inhibitor of the HIV-1 reverse transcriptase and when incorporated into nascent viral DNA causes chain termination. Human cells lack reverse transcriptase and human nuclear DNA polymerases are much less sensitive (by at least 100-fold) to inhibition by ZDV-TP, thus producing a selective effect on viral replication. This mechanism of action is common to all anti-HIV nucleoside analogues.

Adverse effects:

These include the following:

- Dose-dependent bone marrow suppression causing anaemia with reticulocytopenia and granulocytopenia. This occurred in 15% of patients in the original studies with high-dose ZDV. At currently recommended doses, it occurs in only 1–2% of patients;
- Nausea and vomiting;
- Fatigue and headache;
- Melanonychia (blue-grey nail discoloration);
- Lipodystrophy;
- Mitochondrial myopathy (uncommon);
- Hepatic steatosis with lactic acidosis (rarely);
- It is mutagenic and carcinogenic in animals. However, ZDV is used in HIV-positive pregnant women as it reduces HIV maternal–fetal transmission and thus

fetal/neonatal HIV-1 infection and has not been shown to be teratogenic if given to women after the first trimester.

Pharmacokinetics:

Zidovudine is almost totally absorbed (_90%) from the gastro-intestinal tract, it achieves cerebrospinal fluid (CSF) concentrations that are 50% of those in plasma. The ZDV plasma elimination *t*1/2 is one to two hours. About 25–40% of a dose undergoes presystemic metabolism in the liver. The major metabolite (80%) is the glucuronide and approximately 20% of a dose appears unchanged in the urine.

Lamivudine (3TC)[34]

It is a reverse transcriptase inhibitor with a relatively long intracellular half-life (14 h; plasma t1/2 6 h). In combination with zidovudine, lamivudine appears to reduce viral load effectively and to be well tolerated, although bone marrow suppression may be produced. Rarely, pancreatitis may occur. Lamivudine has also been used for treatment of chronic hepatitis B infection, but resistant strains of virus have been reported.

NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITOR:

Tenofovir is the first nucleotide (as distinct from nucleoside) reverse transcriptase inhibitor (NERTI) and is used in combination with NRTIs. It is a derivative of adenosine monophosphate, but lacks the ribose ring. It is phosphorylated sequentially to the diphosphate and then the triphosphate which is a competitive inhibitor of HIV reverse transcriptase. It is adequately absorbed orally and administered once a day (half life 14–17 hours). It is renally eliminated. Tenofovir is well tolerated with few adverse effects (mainly flatulence). Occasional cases of renal failure and Fanconi syndrome have been reported, so it should be used with caution in patients with pre-existing renal dysfunction. Although it is not a CYP450 inhibitor or inducer, it increases the AUC of didanosine and reduces the AUC of atazanavir. Ritonavir and atazanavir increase the AUC of tenofovir. Tenofovir is also active against hepatitis B virus (HBV).

NON-NUCLEOSIDE ANALOGUE REVERSE TRANSCRIPTASE INHIBITORS:

The non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs) are used as part of triple-therapy schedules in combination with nucleoside analogue RT inhibitors (e.g. ZDV/ 3-TC). Agents in this group include efavirenz, nevirapine and delavirdine. Efavirenz is administered orally and causes a marked (_50%) reduction in viral load during eight weeks of therapy. They are synergistic with NRTIs. NNRTIs should only be used in combination therapy due to the rapid development of viral resistance.

Mechanism of action:

Non-nucleoside agents inhibit HIV reverse transcriptase by binding to an allosteric site and causing non-competitive enzyme inhibition, reducing viral DNA production.

Adverse effects:

These include the following:

- Abdominal pain and nausea/vomiting/diarrhoea;
- Lipodystrophy;
- Arthralgia, myalgia;
- Drug–drug interactions: complex effects on other CYP450 metabolized drugs\ (see below);
- Neural tube defects in the fetus.

Pharmacokinetics:

Efavirenz is well absorbed. It has a plasma *t*1/2 of 40–60 hours, is highly protein bound and metabolized by hepatic CYP2B6 _ CYP3A) to its hydroxylated metabolite, which is glucuronidated and excreted in the urine.

Drug interactions:

Efavirenz inhibits CYP3A4, CYP2C9 and CYP2C19 and may reduce the clearance of co-administered drugs metabolized by these isoenzyme systems. Efavirenz autoinduces its own metabolism. In contrast, nevirapine induces CYP3A and thus increases the clearance of drugs metabolized by this isoenzyme.

HIV PROTEASE INHIBITORS:

Compounds in this class include amprenavir, ritonavir, indinavir, lopinavir, nelfinavir, saquinavir, atazanavir and tipranavir . They cause a rapid and marked reduction of HIV-1 replication as measured by a fall of 100- to 1000- fold over 4–12 weeks in the number of HIV RNA copies per mL of plasma. Reductions in viral load are paralleled by increases in CD4 count of approximately 100–150 cells/μL. Resistance is a problem and leads to cross-resistance between protease inhibitors (PIs), so they are used in combination therapy.

Mechanism of action:

These agents prevent HIV protease from cleaving the gag and gag–pol protein precursors encoded by the HIV genome, arresting maturation and blocking the infectivity of nascent virions. The HIV protease enzyme is a dimer and has aspartylprotease activity. Anti-HIV protease drugs contain a synthetic analogue structure of the phenylalanine–proline sequence of positions 167–168 of the gag–pol polyprotein. Thus they act as competitive inhibitors of the viral protease and inhibit maturation of viral particles to form an infectious virion.

Adverse effects:

These include the following:

- Nausea, vomiting and abdominal pain;
- Fatigue;
- Glucose intolerance (insulin resistance or frank diabetes mellitus) and hypertriglyceridaemia;
- Fat redistribution – buffalo hump, increased abdominal girth;
- Drug–drug interactions – complex effects on many other drugs that are hepatically metabolized.

Pharmacokinetics:

Lopinavir is well absorbed with food and 98–99% protein bound (albumin and alpha-1-acid glycoprotein). It undergoes oxidative metabolism by the CYP3A isozyme, with a half-life of five to six hours. The majority of lopinavir is excreted as metabolites in the faeces, with only about 4% appearing in urine. Ritonavir is also well absorbed (bioavailability _60%). It is 60% plasma protein bound and metabolized by CYP3A _ CYP2D6. It has a half life of between three and five hours. Ritonavir inhibits the metabolism of certain CYP3A substrates (and certain drugs metabolized by CYP2D6) and induces its own metabolism. Therefore drug–drug interactions are complex.

Drug interactions:

These are numerous and clinically important; the following list is not comprehensive:

1. Most protease inhibitors are inhibitors of hepatic CYP3A. This leads to reduced clearance and increased toxicity of a number of drugs often causing severe adverse effects (e.g. increased sedation with midazolam, triazolam and excessive hypotension with calcium channel blockers). Protease inhibitors inhibit the metabolism of rifabutin increasing the risk of rifabutin toxicity.

2. Enzyme inducers (e.g. rifamycins – rifampicin/rifabutin or nevirapine) enhance the metabolism of protease inhibitors, making them less effective, producing subtherapeutic plasma concentrations and increasing the likelihood of HIV resistance.

3. Several protease inhibitors reduce gastro-intestinal metabolism (by CYP3A) and luminal transport (via P-gp/MDR1) of co-administered protease inhibitors, thereby increasing plasma concentrations. Combining two agents from this group is called ‘boosted protease inhibitor’ therapy, e.g. lopinavir is available combined with low-dose ritonavir; ritonavir inhibits CYP3A and P-glycoprotein (MDR1) increasing the bioavailability of lopinavir. The same principle applies if saquinavir/ low-dose ritonavir or amprenavir/low-dose ritonavir are combined.

FUSION INHIBITORS:

Currently, enfuvirtide is the only available HIV fusion inhibitor. This agent is reserved for HIV patients who have evidence of progressive HIV replication despite HAART therapy. It is a 36 amino acid peptide analogue of part of the transmembrane region of gp41 that is involved in the fusion of the virus particle with the host cell membranes. It is given subcutaneously on a twice daily basis.

Mechanism of action:

The peptide enfuvirtide blocks the interaction between the HIV gp41 protein and the host cell membrane by binding to a hydrophobic groove in the N36 region of gp41. Due to this unique mechanism of action, enfuvirtide is active against HIV which has developed resistance to HAART. Resistance to enfuvirtide can arise by mutations in its gp41 binding site.

Adverse effects:

These include:

- Injection site reactions – pain, erythema, induration (98% of patients) and nodules; approximately 5% of patients discontinue therapy because of these local skin reactions;
- lymphadenopathy;
- flu-like syndrome;
- eosinophilia;
- biochemical hepatitis.

Pharmacokinetics:

Enfuvirtide is well absorbed after subcutaneous administration and is distributed in the plasma volume, with 98% bound to albumin. The plasma *t*1/2 is three to four hours. The major route of clearance is unknown.

Drug interactions:

Enfuvirtide is not known to cause drug–drug interactions with other anti-HIV drugs.

CHANGING ANTI-HIV THERAPY FOR TREATMENT FAILURE AND/OR RESISTANCE:

A change in anti-HIV therapy may be required because of treatment failure, adverse effects, poor compliance, potential drug–drug interactions or current use of a suboptimal regimen. Viral resistance to NRTIs and NNRTIs and protease inhibitors may cause treatment failure. Reduced susceptibility of HIV-1 isolates to NRTIs/NNRTIs and PIs is developing. Genetic testing of the HIV genome for mutations leading to drug resistance in isolates from individual patients is becoming

more widely available and may guide therapy. Resistance to ZDV emerges more quickly and to a greater degree in the later stages of the disease. Progressive stepwise reductions in susceptibility of the HIV reverse transcripase (RT) correlate with the acquisition of mutations in the gene for the RT protein. In the case of ZDV, the only cross-resistance is to other nucleosides with the 3_-azido side-chain and therefore such isolates are still sensitive to 3-TC, d4T/ddI. Future prospects include more potent protease inhibitors, novel entry inhibitors e.g. maraviroc, HIV-integrase inhibitors e.g. raltegravir and effective anti-HIV vaccines.

2.Controlled release Dosage Form:

This is reflected by the fact that well over 80% of the drugs in United States that are formulated to produce systemic effects are marked as oral dosage forms Compared with other oral dosage forms, tablets is manufacturer's choice because of their

- Relatively low cost of Manufacturing
- Packaging
- Shipping
- Increased stability and virtual tamper resistance.

2.1 TABLETS[1]

Tablets are solid preparations each containing a single dose of one or more active substances and usually obtained by compressing uniform volumes of particles. The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the preparation in the digestive tract, colouring matter authorised by the competent authority and flavouring substances.

ADVANTAGES OF TABLET[2,3,4]

Some of the potential advantages of tablets are as follows .They are the unit dosage forms having greatest capabilities amongst all the oral dosage form for the dose precision and least content variability.

- Low cost amongst all the oral dosage forms.
- They are the lightest and the most compact amongst all the oral dosage form.
- Product identification requires no additional processing steps when employing an embossed or monogrammed punch face.
- Provides greatest ease of swallowing with the least tendency for hang up above the stomach.
- They are easiest and cheapest for packaging and transportation.
- They lend themselves to certain special release profile products such as enteric or delayed release products.
- Tablets are better suited to large-scale production than other unit oral dosage forms.
- They have the best-combined properties of chemical, mechanical, microbiological stability amongst all the oral dosage forms.
- Emergencies supplies of the drug can be conveniently carried by the patient.

TYPES OF TABLETS

Tablets are divided into classes based on their route of administration and their function.

I. Tablets administered orally

    i. Compressed tablets

- Sugar coated tablets
- Film-coated tablets
- Enteric-coated tablets
- Chewable tablets
- Controlled release tablets

ii. Multiple compressed tablets

- Layered tablets
- Press coated tablets

II. Tablets administration in oral cavity

i. Buccal and sublingual tablets

ii. Lozenges and troches

iii. Dental cores

III. Tablet administered via other routes

i. Implants

ii. Compressed suppositories or Inserts

IV. Tablets administered in solution form

i. Effervescent tablets

ii. Dispensing tablets

iii. Hypodermic tablets

iv. Tablets triturates

2.2 AN INTRODUCTION TO CONTROLLED RELEASE DOSAGE FORMS[6&8]:

The R&D laboratories have brought the concept, “Development & optimization of rate controlled drug delivery” The science and technology of rate controlled administration of the drug substance has generated wide application for future to come. Many categories of drug substances have been exploited as a candidate for such type of drug delivery systems.

Curing of the patient suffering from any kind of pain and inflammation is also favourable through such type of drug delivery system. The oral route of administration has been used for both conventional and novel drug delivery systems. There are many obvious reasons for the same, not the least of which would include acceptance by the patient and ease of administration. The types of sustained and controlled-release systems employed for oral administration include virtually every currently known theoretical mechanism for such application. This is because there is more flexibility in dosage design, since constraints, such as sterility and potential damage at the site of administration are minimized. Because of this, it is convenient to discuss the different types of dosage forms by using those developed for oral administration as initial examples.

Most orally administered drug, targeting is not a primary concern, and it is usually intended for drugs to permeate to the general circulation and perfuse to other body tissues for this reason, sustained-release system are employed. It is assumed that increase circulating blood levels which, in turn, promotes greater concentrations of drug at the site of action. If toxicity is not as issue, therapeutic levels can thus be extended. In essence, drug delivery by these systems usually depends on release from some type of dosage form, permeation though the biological milieu and absorption through an epithelial membrane to the blood. There are a variety of both physicochemical and biological factors that plays important roll in the design of such systems.

To maintain the drug concentration within therapeutic range, it is necessary to take these conventional dosage forms frequently. This may lead to the peak and valley type of drug concentration in the body. It is explained in the following graphical presentation.

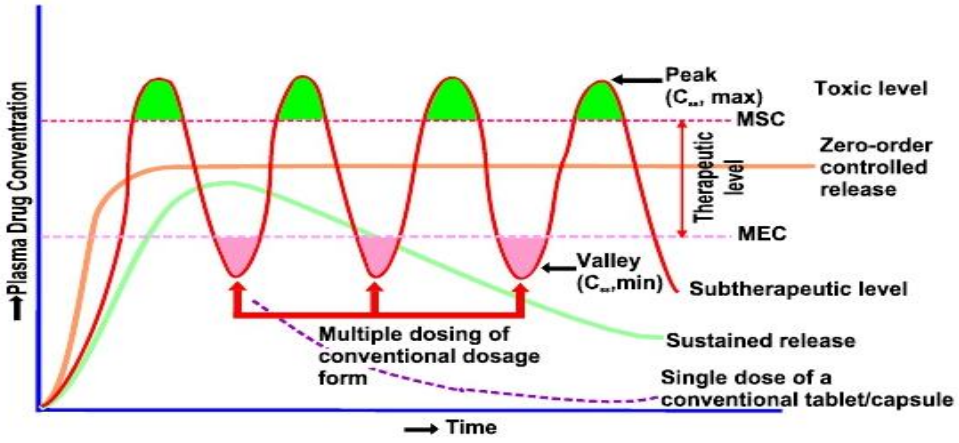


Fig. No.3- A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations.

The goal of any drug delivery system is thus to provide a therapeutic amount of drug to the proper site in the body, secondly to achieve promptly and then maintain the desired drug concentration for therapeutic benefits. The idealized objective points to the two aspects most important to the drug delivery namely, spatial placement and temporal delivery of drug. Spatial placement relates to the targeting of a drug to specific organ or tissue while temporal delivery refers to controlling the rate of drug delivery to target tissue. An appropriately designed controlled release delivery system can be a major advance towards solving these two problems.

There are many definitions of controlled release but the simplest definition is “Any drug or dosage form or medication that prolongs the therapeutic activity of drug’’. The overall objective is that, once the drug-carrier material has been injected or otherwise implanted or taken orally into the body, the drug is released at a predetermined rate for some desired period of time. Controlled release technology is

relatively new field and as a consequence, research in this field has been extremely fertile and has produced many discoveries.

Several terms have been used to describe the various types and modes of action intended to provide long duration of drug activity. Unfortunately, the terms have been applied loosely and are interchanged invariably so that today there is no consistent nomenclature for the prolonged action products available in the market. Several nomenclatures have been utilized synonymously to describe controlled release medications. Some of these are: continuous release (CR), depot release (DR), slow release (SR), long acting (LA), long lasting (LL), long term release (LTR), prolonged action (PA), controlled release (CR), extended release (ER), gradual release (GR) etc. The United States Pharmacopoeia (U.S.P.) has adopted the term extended release whereas British pharmacopoeia (B.P.) has adopted the term slow release. The Indian pharmacopoeia (I.P.) does not make any reference to controlled release products. The food and drug administration (F.D.A.) of United States has adopted the term prolonged release. Various other designations such as smart release, targeted release, repository release, intelligent release, protracted release, spaced release have been assigned to describe sustained release systems. However, the recent literature survey indicates that as on today the most widely used terms are controlled release and sustained release.

The aim of controlled release delivery of drugs, in a general way is to modify the normal behavior of drug molecule in physiological environment. It can lead to the following: -

1. Controlling drug action at a predetermined rate by maintaining a relatively constant effective drug level in the body with concomitant minimization of undesirable side effects.

2. Localization of drug activity by spatial placement of a control release system adjacent to or within the diseased tissue or organ.

3. Targeting drug action by using specific carriers or chemical derivatives to deliver drugs to particular target cell type

ADVANTAGES OF CONTROLLED RELEASE SYSTEM [4&7.]

Therapeutic efficacy and safety of controlled release dosage formulations attained as a result of providing a nearly constant pharmacological response, there by avoiding the normal peack and valley pattern associated with multiple dosing of conventional drug product**.**

- Control drug release improves patient convenience.
- Decrease incidences of adverse drug reactions.
- Increased safety margin of high potency drug dye to better control of plasma levels.
- Maximum utilization of drug enabling reduction total amount of dose administered.
- Controlled release dosage form will provide therapeutic concentration of the drug in the blood that is maintained throughout the dosing interval.
- Reduction in health care costs through improved therapy, shorter treatment period less frequency of dosing and reduction in personnel time to dispense administer and monitor patients.

2.3 PHYSIOCHEMICAL PROPERTIES OF THE DRUG SUBSTANCE SUITABLE FOR ORAL CONTROLLED RELEASE DOSAGE FORMS[8&32]

Some characteristics make a drug more suitable for controlled release dosing, such as-

1. Elimination half-life between 2 to 8 hours.
2. Broader therapeutic index.
3. Moderate unit dose
4. Significant extent of absorption in GIT.

5. Optimum solubility characteristics.

6. Minimal first-pass clearance.

2.4 FACTORS INFLUENCING THE PERFORMANCE OF ORAL CONTROLLED RELEASE PRODUCTS[8&32]:

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products in different dosage forms. Irrespective of their mode of delivery (immediate, sustained or controlled release) and the design of dosage forms (either solid or liquid) they must be developed within the intrinsic characteristics of GI physiology. Therefore a fundamental understanding of pharmacokinetics, pharmacodynamics and formulation design is essential to achieve a systematic approach to the successful development of an oral pharmaceutical dosage form.

A number of variables such as drug properties, route of delivery, target sites, duration of therapy, the disease state and patient variables must be considered. The formulation and performance of sustained release products are greatly influenced by the physicochemical and biological properties of drug.

2.5 PHYSICOCHEMICAL PROPERTIES OF A DRUG AFFECTING DRUG PRODUCT DESIGN AND PERFORMANCE[6,8&32.]

1. DOSE SIZE:

Single oral dose greater than 200mg are poor candidates for the oral controlled release products as the maintainence dose will be unacceptably large depending on the density of the drug, duration of intended prolongation and type of controlling mechanism.

2. AQUEOUS SOLUBILITY:

Since drugs must be in solution before they can be absorbed, compounds with very low aqueous solubility usually suffer oral bioavailability problems because of limited gastrointestinal transit time of the undissolved drug particles and limited solubility at absorption site e.g. warfarin and griseofulvi Hence diffusion controlled systems will be poor choice for such drugs.

Drugs having very high aqueous solubility and drugs exhibiting pH-dependent solubility may also pose problems for their incorporation in oral controlled release system.

3. DRUG STABILITY:

Since most of the controlled release preparations are designed to release the drug over the entire length of GI tract, drugs that are unstable in gastric or intestinal environment are unsuitable for sustained release dosage form.

4. pK_a:

The uncharged form of a drug is preferentially absorbed through many body tissues. The release of an ionisable drug from a controlled release unit should hence be programmed in accordance with variation in pH of the gastro-intestinal tract so that the amount of preferentially absorbed uncharged species and the plasma levels of the drug will be approximately constant throughout the duration of action.

2.6 CLASSIFICATION OF POLYMERIC DRUG CONTROLLED RELEASE DRUG DELIVERY SYSTEMS[32]:

Once a drug candidate is thoroughly investigated and found suitable, it is needed to select, the controlled release technology that best fits the intended application and the basic physical form of device and the rate-controlling polymer matrix to be used.

The various controlled release polymeric systems can be classified depending upon the mechanism of controlling the drug release, as follows:

I. Chemically controlled systems

i) Biodegradable systems

ii) Drug –polymer conjugates

II. Diffusion-controlled systems

i) Membrane-reservoir systems

ii) Matrix systems

I. CHEMEMICALLY-CONTROLLED SYSTEMS:

i) BIODEGRADABLE SYSTEMS:

In this system, the matrix-forming polymer contains hydrolytically or enzymatically labile bonds and drug is uniformly dissolved or dispersed in this matrix. As the polymer erodes by hydrolysis or enzymatic cleavage, the drug is released to the surrounding environment. The erosion process has a direct effect on drug release.

ii) DRUG-POLYMER CONJUGATES:

This system involves drug molecules chemically bonded to a polymer backbone. The drug will be released through hydrolytic or enzymatic cleavage of these bonds. The attachment of drugs to macromolecular carriers alters their rate of excretion from the body and provides the possibility for sustaining the release over a prolonged period.

II. DIFFUSION-CONTROLLED SYSTEMS [7,21&24]:

i) MEMBRANE-RESERVOIR SYSTEM:

The kinetics of drug release from membrane-reservoir systems generally follows either a solution diffusion mechanism or an osmotic pumping mechanism.

In the solution-diffusion mechanism, the drug transport occurs by first dissolving in the reservoir membrane at one interface followed by diffusion down a chemical potential gradient across this membrane and eventually released from the second interface into the external medium. Such solution-diffusion mechanism is typically observed in non-porous membranes.

In the osmotic pumping mechanism, a semi permeable membrane is utilized to regulate the osmotic permeation of water/GI fluid. The rate of osmotic water influx and therefore the rate of drug delivery will be constant as long as a constant thermodynamic activity gradient is maintained across the membrane. The delivery rate from such devices is generally regulated by osmotic pressure of the drug core formulation and by the water permeability of the semipemeable membrane.

ii) MATRIX SYSTEMS:

Matrix system is one of the least complicated approaches to manufacture the sustained release dosage forms. Matrix systems can be classified based on the mechanism of controlling the drug release as follows:

a. Matrix diffusion

b. Polymer erosion

c. Polymer swelling

a. MATRIX DIFFUSION:

Historically, the most popular diffusion-controlled delivery system has been the matrix system. However, the inherent drawback of the matrix system is its first-order release behaviour with continuously diminishing release rate. This is a result of the increasing diffusional resistance and decreasing area at the penetrating diffusion front.

The kinetics of drug release from homogeneous dispersed-drug matrix devices (which acts as a diffusional medium) can be given by Higuchi’s equation,

$$Q = [D\ t\ (2A - C_s)\ C_s]^{½}$$

where Q = amount of drug released after time t,

D = diffusivity of the drug in the homogenous matrix media,

C_s = the solubility of the drug in the matrix substanc

A = surface area of drug particle.

b. POLYMER EROSION:

The release of a dissolved drug or dispersed drug from an erodible polymer matrix can be controlled by a variety of mechanisms ranging from hydrolysis/enzymatic cleavage to swelling and dissolution. The situation where polymer erodes by a purely heterogeneous process, viz.surface erosion, is of special interest because the drug release from such devices having constant geometry will be at constant rate.

c. POLYMER SWELLING:

Swelling phenomena are generally encountered in both the hydrophilic and hydrophobic polymer matrices during the release of entrapped water-soluble drug in an aqueous environment.

Among different technologies use in controlled drug delivery hydrophilic matrix system are the most popular because of the simplicity of formulation, ease of manufacturing low cost , FDA acceptance and applicability to drug with wide range of solubility.

2.7 FORMULATION OF ORAL CONTROLLED RELEASE DOSAGE FORMS:

Oral polymeric matrices are commonly employed to achieve controlled release of drugs when a hydrophilic matrix is placed in an aqueous medium the hydrophilic colloid component swells to form a gelatinous surface layer this then controls the diffusion of water into the matrix release of drugs from such a system is governed by two mechanism (I) diffusion of water-soluble drug through the gel

layer and (ii) release of a water soluble or water insoluble drug by erosion of the outer gel layer as it becomes well-hydrated within the hydrated surface layer of the matrix the core remains drug acting as a non-releasing reservoir of drug polymer.

Extensive research has been carried out in the field of development of oral controlled release dosage forms, which are mostly in the form of tablets and capsules. A wide variety of materials has been tried for controlling/sustaining drug release and these include wax, carnauba wax, stearic acid, stearyl alcohol, synthetic polymers such as polyvinyl alcohol, polyvinyl chloride, polyvinyl pyrolidone, and acrylates; hydrocolloids and gums such as methyl cellulose, corboxy methyl cellulose, hydroxy propyl methyl cellulose, xanthan gum, guar gums, silicones and related polymers and also ion exchange resins.

The technologies adopted for design of oral extended release dosage forms are barrier coating, embedment matrix, micro encapsulation, use of ion exchange resins and Osomotically-controlled devices.

2.8 BIOMATERIALS FOR DELIVERY SYSTEMS[15]

A range of materials have been employed to control the release of drugs and other active agents. The earliest of these polymers were originally intended for other, nonbiological uses, and were selected because of their desirable physical properties, for example:

Poly(urethanes) for elasticity.

Poly(siloxanes) or silicones for insulating ability.

Poly(methyl methacrylate) for physical strength and transparency.

Poly(vinyl alcohol) for hydrophilicity and strength.

Poly(ethylene) for toughness and lack of swelling.

Poly(vinyl pyrrolidone) for suspension capabilities.

To be successfully used in controlled drug delivery formulations, a material must be chemically inert and free of leachable impurities. It must also have an appropriate physical structure, with minimal undesired aging, and be readily processable. Some of the materials that are currently being used or studied for controlled drug delivery include

Poly(2-hydroxy ethyl methacrylate).

Poly(N-vinyl pyrrolidone).

Poly(methyl methacrylate).

Poly(vinyl alcohol).

Poly(acrylic acid).

Polyacrylamide.

Poly(ethylene-co-vinyl acetate).

Poly(ethylene glycol).

Poly(methacrylic acid).

However, in recent years additional polymers designed primarily for medical applications have entered the arena of controlled release. Many of these materials are designed to degrade within the body, among them

Polylactides (PLA).

Polyglycolides (PGA).

Poly(lactide-co-glycolides) (PLGA).

Polyanhydrides.

Polyorthoesters.

Originally, polylactides and polyglycolides were used as absorbable suture material, and it was a natural step to work with these polymers in controlled drug

delivery systems. The greatest advantage of these degradable polymers is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. However, biodegradable materials do produce degradation by-products that must be tolerated with little or no adverse reactions within the biological environment.

These degradation products—both desirable and potentially nondesirable—must be tested thoroughly, since there are a number of factors that will affect the biodegradation of the original materials. The most important of these factors are shown in the box below—a list that is by no means complete, but does provide an indication of the breadth of structural, chemical, and processing properties that can affect biodegradable drug delivery systems.

FACTORS AFFECTING BIODEGRADATION OF POLYMERS[18]

- Chemical structure.
- Chemical composition.
- Distribution of repeat units in multimers.
- Presents of ionic groups.
- Presence of unexpected units or chain defects.
- Configuration structure.
- Molecular weight.
- Molecular-weight distribution.
- Morphology (amorphous/semicrystalline, microstructures, residual stresses).
- Presence of low-molecular-weight compounds.
- Processing conditions.
- Annealing.
- Sterilization process.
- Storage history.

- Shape.
- Site of implantation.
- Adsorbed and absorbed compounds (water, lipids, ions, etc.).
- Physicochemical factors (ion exchange, ionic strength, pH).
- Physical factors (shape and size changes, variations of diffusion coefficients, mechanical stresses, stress- and solvent-induced cracking, etc.).
- Mechanism of hydrolysis (enzymes versus water).

2.9 CLASSIFICATION OF POLYMERS USED IN CONTROLLED RELEASE DRUG DELIVERY SYSTEMS[18&20]:

Polymers are complex and giant molecules known as macromolecules consisting of many repeating units and are formed by process called as polymerization.

In general polymers can be classified in to three distinct categories i.e. natural, synthetic and semi synthetic

Natural polymers include nucleic acids, polysaccharides, complexes of proteins and polysaccharides. Synthetic polymers belong to classes of polyesters, polyurethanes, polyamides, polycarbonates, polyolefins, polyvinyls, acrylics etc.

Pharmaceutical formulators have employed natural polymers for hundreds of years and more recently the chemical modifications of some of these have provided a range of materials with improved performance. The choice regarding polymers used as release retardants for drug delivery systems depends upon their availability, cost, mechanical and physical properties and regulatory acceptance. The polymers used in extended release dosage forms can be classified as

2.9.1 CLASSIFICATION OF POLYMERS USED IN SUSTAINED RELEASE DRUG DELIVERY SYSTEMS ACCORDING TO THEIR CHARACTERISTICS[20]:

Polymers can also be classified on the basis of their interaction with water into:

(a) Non-biodegradable hydrophobic polymers

(b) Hydrogels

(c) Soluble polymers

(d) Biodegradable polymers

2.9.2 CHARACTERISTICS OF IDEAL POLYMER SYSTEM

- It should be chemically inert and free from leachable impurities.
- It should have good mechanical strength.
- It should be non-toxic and compatible with the environment.
- It should be easy and inexpensive to fabricate.
- It should be easily sterilized.
- It should demonstrate acceptable shelf life.

In addition to carrier systems containing single polymeric systems, researchers are working on carrier systems containing block copolymers. These are the polymers formed through polymerizatioin of two or more monomers. These networks when composed of hydrophilic and hydrophobic monomers are called polymer micelle. These micelles are suitable for enclosing individual drug molecules. Their hydrophilic outer shells help to protect the cores and their contents from chemical attack by the aqueous medium. Most micelle based systems are formed from poly(ethylene oxide)-bpolypropylene-b-poly(ethylene oxide) triblock

network. In one of the studies carried out, micelles were used as a hydrophobic (doxorubicin) anticancer agent. The results revealed that when dose of drug intravenously was given, the system could withstand the body's normal blood circulation and effectively deliver the medication to a solid cancerous tumor.

2.9.3 DRUG RELEASE MECHANISMS FOR POLYMERIC DRUG DELIVERY[8&32]

Two broad categories of polymer systems have been studied. The reservoir device involves the encapsulation of a drug within a polymer shell, while the matrix device describes a system in which a drug is physically entrapped within a polymer network.

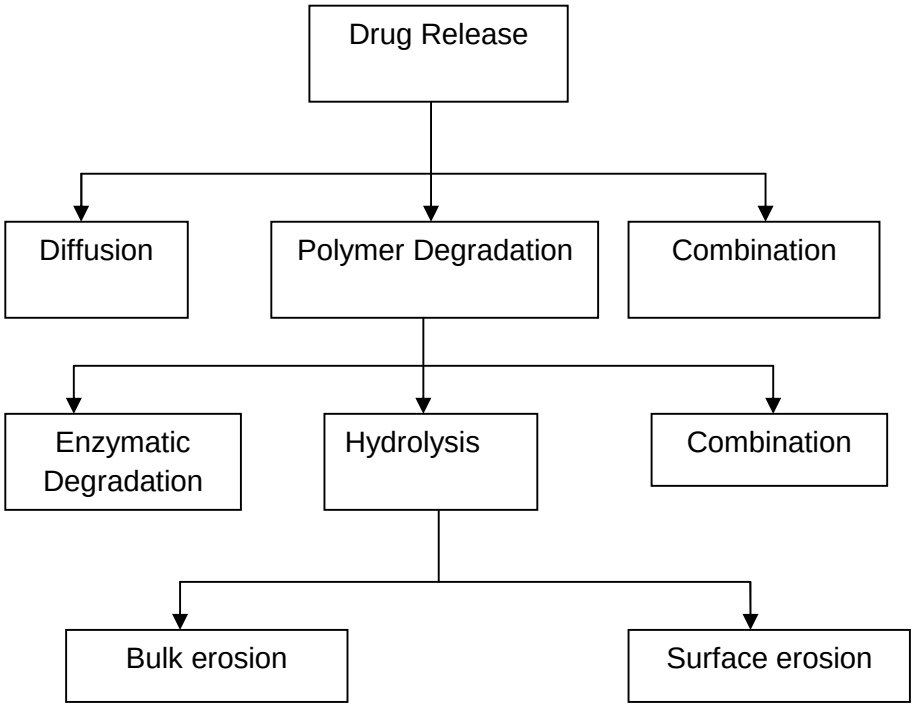


Fig.No.4:- Possible drug release mechanisms for polymeric drug delivery.

As shown in Fig.No.2 the drug will be released over time either by diffusion out of the polymer matrix or by erosion (due to degradation) of the polymer or by a combination of two mechanisms. Reviews have been presented on the mechanisms and the mathematical aspects of release of drug from polymer matrices.

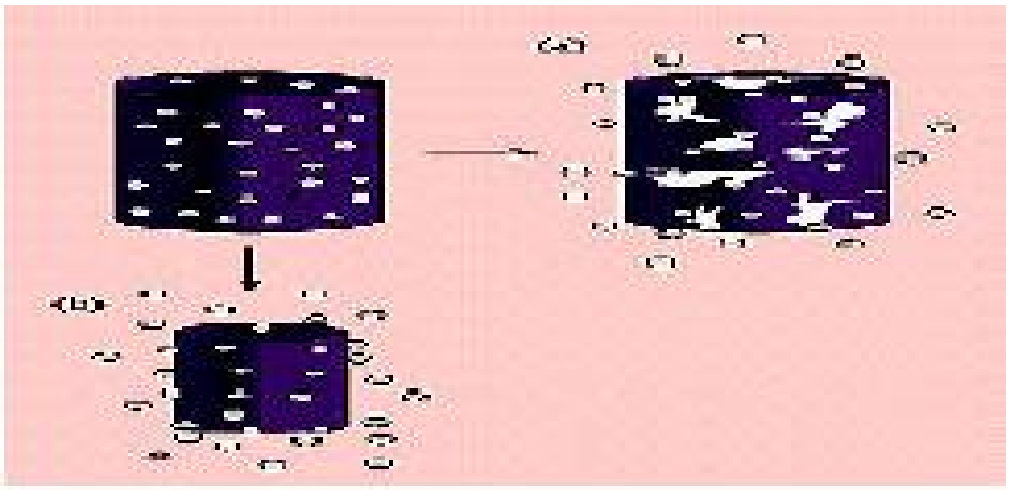


Fig.No.5:- Drug delivery from (a) bulk eroding and (b) surface eroding biodegradable system.

Table No.1:- List of polymers and their application[20].

POLYMER	**APPLICATION AND COMMENT**
NATURAL POLYMERS	
Proteins and protein-based polymers	Absorbable, biocompatible, nontoxic, naturally available, typically elastic materials used as implants and in tissue engineering.
Collagen	Absorbable sutures, sponge wound dressing, drug delivery microspheres.
Albumin	Used in cell and drug microencapsulation.
Poly (amino acids)	Nontoxic, nonantigenic and biocompatible. Used as oligomeric drug carriers.
Polysaccharides and derivatives	
From vegetable sources	
Carboxymethyl cellulose	Cell immobilization via a combination of ionotropic gelation and polyelectrolyte complex formation (e.g. with chitosan), in

	drug-delivery systems and dialysis membranes.
Cellulose sulphate	Component of polyelectrolyte complexes for immunoisolation.
Agarose	Largely used as supporting materials in clinical analysis and as an immobilization matrix.
Alginate (marine sources, algae)	Excellent gel-formation properties, biocompatible, microstructure and viscosity are dependent on the chemical composition (batch-to-batch variations). Used as immobilization matrices for cells and enzymes, controlled release of bioactive substances, injectable microcapsules for treating neurodegenerative and hormone-deficiency diseases.
Carrageenan	Excellent thermoreversible properties. Used for microencapsulation.
From human and animal sources	
Hyaluronic acid	Excellent lubricant, potential therapeutic agent.
Heparin and heparin-like	Antithrombotic and anticoagulant.
Microbial polysaccharides	
Microbial polysaccharides	Microbial polysaccharides.
Chitosan and its derivatives Biocompatible, nontoxic, excellent gel- and film-forming ability,	Natural polymer. Widely used in controlled-delivery systems.

SYNTHETIC POLYMERS **Aliphatic polyesters**	
Poly (lactic acid), poly (glycolic acid) and their copolymers	Used in sutures, drug-delivery systems and in tissue engineering. Biodegradable, often copolymerized to regulate degradation time.
Poly (hydroxy butyrate), poly (e-caprolactone) and copolymers	Biodegradable, used as a matrix for drug-delivery systems, cell microencapsulation. Properties can be changed by chemical modification, copolymerization and blending.
Polyamides (nylons)	Sutures, dressing, haemofiltration membranes.
Polyanhydrides	Biodegradable, useful in tissue engineering and for the release of the bioactive molecules.
Poly (ortho esters)	Surface-eroding polymers. Application in sustained drug delivery, ophthalmology.
Poly (cyano acrylates)	Biodegradable, depending on the length of the alkyl chain. Used as surgical adhesives and glues, potentially used in drug delivery.
Polyphosphazenes	Can be tailored with versatile side-chain functionality. Made into films and hydrogels. Applications in drug delivery.
Can be tailored with versatile side-chain functionality. Made into films and hydrogels. Applications in drug delivery.	Good elastomeric properties. Can be tailored by varying the starting materials. Used in permanently implanted medical devices (prostheses, vascular grafts), catheters and drug delivery systems. Initial candidates for the artificial heart.
Polyethylene (low density)	Sutures, catheters, membranes, in surgery.
Poly (vinyl alcohol)	Gels and blended membranes are used in drug delivery and cell immunoisolation.

Poly (ethylene oxide)	Highly 'biocompatible'. Different polymer derivatives and copolymers have been utilized in a variety of biomedical applications.
Poly (hydroxyethyl methacrylate)	Hydrogels have been used as soft contact lenses, for drug delivery, as skin coatings and for immunoisolation membranes.
Poly (methyl methacrylate)	This and its copolymers are used as dental implants and in bonereplacement.
Poly (tetrafluoroethylene) (Teflon®)	Vascular grafts, clips and sutures, coatings.
Polydimethylsiloxanes	A silicone. Implants in plastic surgery, orthopedics, blood bags and pacemakers.

Environmentally responsive, synthetic polymers	
Poly (ethylene oxide-b-propylene oxide)	Surfactants with amphiphilic properties; used in protein delivery, skin treatments.
Poly (vinyl methyl ether)	Nontoxic, temperature-sensitive polymer; excellent shapememory properties.
Poly (N-alkylacrylamides)	Temperature-sensitive gels whose lower critical solution temperature can be adjusted via co-monomer incorporation.

2.12 TABLET PRODUCTION METHOD[2&16].

Tablets are manufactured by wet granulation, dry granulation or direct compression method.

2.12.1 WET GRANULATION

Wet granulation is the process in which a liquid is added to a powder in a vessel equipped with any type of agitation that will produce agglomeration or granules. These granules after drying are compressed to form tablets.

This method has more operational manipulations, and is more time-consuming than the other methods. The wet granulation method is not suitable for drugs, which are thermolabile or hydrolysable by the presence of water in the liquid binder.

ADVANTAGES OF WET GRANULATION

- Traditional method, works well for many drugs because it imparts compressibility.

- ❖ Useful for fluffy powders that don't flow well or mix well, thus improving uniformity important for potent (low dose) drugs.
- ❖ Wide range of available excipients.

DISADVANTAGES OF WET GRANULATION

Not useful for moisture sensitive or heat sensitive drugs.

- ❖ Need to use a binder in the excipients mix.
- ❖ Extra steps for drying and labour, material losses susceptible to
- ❖ Contamination, time consuming and expensive.

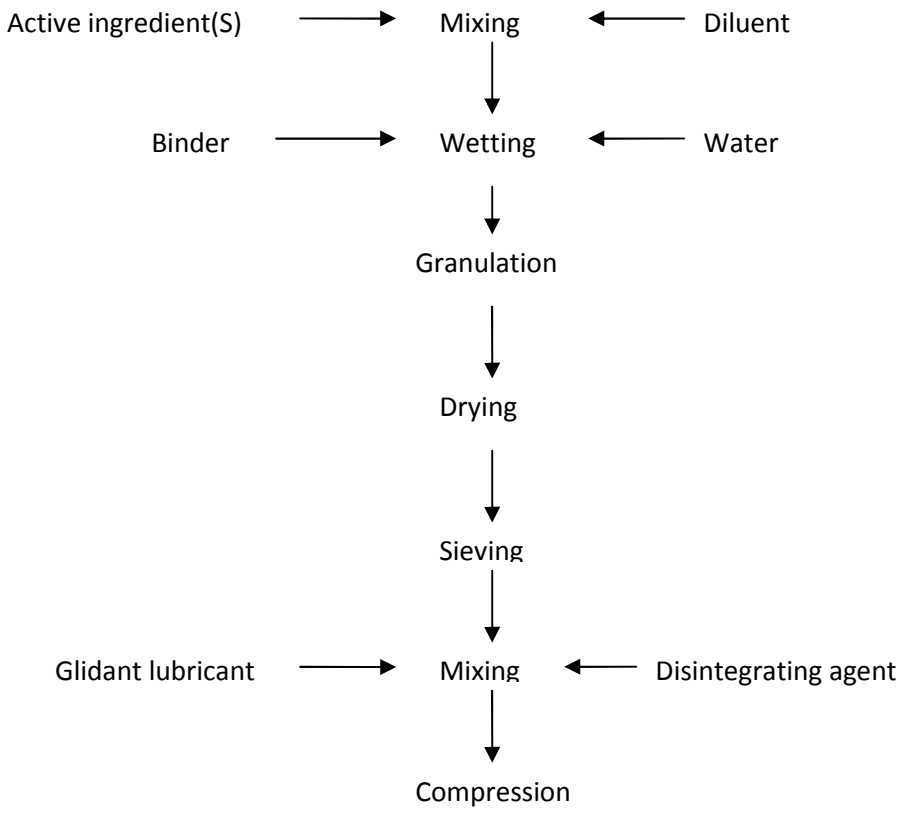


Fig.No.6:- Wet granulation process of tablet.

2.12.2 DRY GRANULATION

In this technique, there is no use of liquids. The process involves the formation of slugs. Then the slugs are screened or milled to produce granules. The granules formed are then compressed to form tablets**.**

ADVANTAGES OF DRY GRANULATION

- Useful for moisture-sensitive drugs.
- Fewer steps and equipment than wet granulation.
- Less loss of materials e.g. transfer steps (mixer-granulators).
- May be less expensive.
- Less time-consuming.

DISADVANTAGES OF DRY GRANULATION

- Lots of force used to compress the dry mixture.
- This energy input can alter crystal form (polymorphism of drugs can affect their therapeutic effects).
- Makes very hard granules –disintegration/dissolution can be affected (this can affect the pharmacokinetic of the drug).
- May generate static – poor powder flow, explosion risk with some mixtures (need special safety equipment, space, money used)

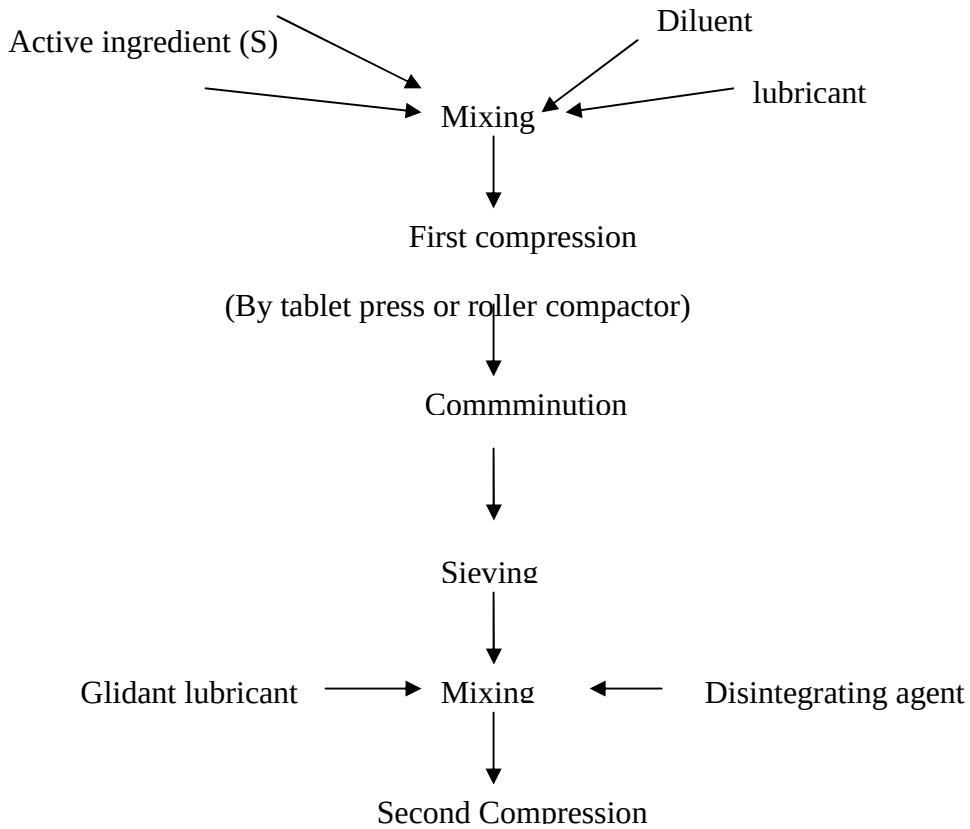


Fig.No.7:- Dry granulation process of tablet manufacture.

2.12.3 DIRECT COMPRESSION[16]

The term direct compression is used to define the process by which tablets are compressed directly from powder blends of active ingredient and suitable excipients, which will flow uniformly in the die cavity & forms a firm compact. Direct compression methods are very popular because it reduces the number of steps involved and the materials required.

ADVANTAGES OF DIRECT COMPRESSION METHOD

- This process is more economical, it requires fewer manufacturing steps, less processing time & thus reduces labour cost & less process validation.
- There is an optimization of tablet disintegration, in which each primary
- drug particle is liberated from the tablet mass & is available for dissolution.

- Disintegrating agents like starch are more effective when processed by direct compression than wet granulation technique.

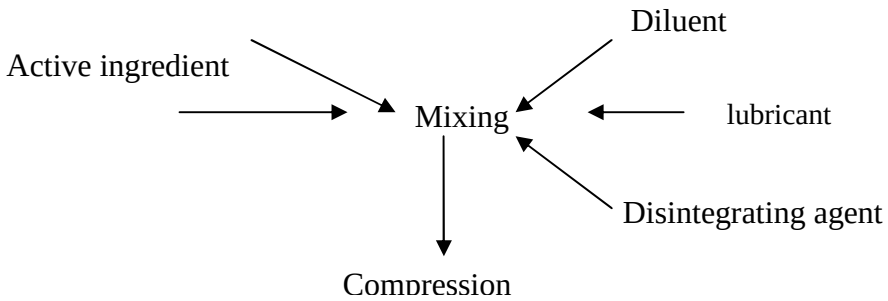


Fig.No.8:- Direct compression process of tablet manufacture.

Chapter II

Literature Review

2. REVIEW OF LITERATURE

Punna Rao Ravi[35] ***et al.,*** Formulate the Oral controlled release matrix tablets of zidovudine were prepared using different proportions and different viscosity grades of hydroxypropyl methylcellulose. The effect of various formulation factors like polymer proportion, polymer viscosity and compression force on the in vitro release of drug were studied.

R.K.Kar[36] ***et al.,*** Formulate oral controlled release matrix tablets of Zidovudine (AZT) in order to improve efficacy and better patient compliance. Tablets were prepared by direct compression method using various proportion of hydrophilic polymer viz; Eudragit RS100 and RL100 along or in combination with hydrophobic polymer ethyl cellulose. In vitro release studies were performed using USP type I apparatus (rotary basket type).

Amit.S.Yadav[37] ***et al.,*** Studied design oral controlled release zidovudine matrix tablets by using hydroxyl propyl methyl cellulose polymer (HPMC) as rate controlling factor and to evaluate drug release parameters as per various release kinetic models. The tablets were prepared by wet granulation method. The granules were evaluated for angle of repose, loose bulk density, tapped bulk density and compressibility index, shows satisfactory results.

P Narayana Raju[38] ***et al.,*** The matrix tablets were prepared by using Eudragit L 100, Poly ethylene oxide and Carbopol 971 P. The granules of Zidovudine using above polymers were prepared by direct mixing, wet granulation with water and wet granulation with IPA. This study gives an idea about the feasibility of granulation process for the preparation of the Zidovudine matrix tablets with different polymers.

Nandita G[39] ***et al.,*** The technologies behind oral drug delivery have emerged from the mainstream pharmaceutical industry and have become influential

forces in their own right, as evidenced by the burgeoning “drug delivery companies” that are at the forefront of innovation and hold their own niche market.

G.A. Green[40] *et al.,* Controlled-release (CR) medications offer many clinical and convenience advantages for patients as compared to immediate-release (IR) formulations. Among these are reductions of fluctuations in drug concentration and adverse side effects, increased compliance and a more convenient dosing regimen. The drawback however is the lack of dose flexibility. Many solid oral dosage CR medications are unsuitable for splitting due to the risk of absorption of a large amount of drug into the blood stream if the drug delivery system is compromised.

P.Venkatesh[41] *et al.,* A high-performance liquid chromatographic method was developed and validated for the determination of two antiretroviral drugs viz. Zidovudine and Lamivudine in combined pharmaceutical tablets. The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined.

Amit.S.Yadav[42] *et al.,* Investigate an attempt was made to formulate the oral controlled release zidovudine matrix tablets by using Guar gum as rate controlling polymer and to evaluate drug release parameters as per various release kinetic models. The tablets were prepared by wet granulation method.

Punna Rao Ravi[43] *et al.,* Design oral controlled release matrix tablets of lamivudine using hydroxypropyl methylcellulose (HPMC) as the retardant polymer and to study the effect of various formulation factors such as polymer proportion, polymer viscosity, and compression force on the in vitro release of drug.

G.Deepali[44] *et al.,* Developed A rapid, simple, accurate, and economical spectrophotometric method has been developed and validated for the assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredients (API) and in its tablet formulation. The analysis is based on the UV absorbance maxima at about 270nm wavelength of lamivudine, using methanol as solvent.

Ravi PR[45] *et al.,* Designed oral controlled release (CR) matrix tablets of zidovudine (AZT) using hydroxypropyl methylcellulose (HPMC), ethyl cellulose (EC) and carbopol-971P (CP) and to study the effect of various formulation factors on in vitro drug release. Release rate decreased with increase in polymer proportion and compression force. The release rate was lesser in formulations prepared using CP (20%) as compared to HPMC (20%) as compared to EC (20%).

Emeje M[46] *et al.,* Designed Oral sustained release matrix tablets of zidovudine (ZDV) were prepared using different types, proportions and blends of carbopol 71G (C71) and a plant gum obtained from Abelmoschus esculentus (AEG). The effect of various formulation factors like polymer proportion, polymer type and pH of the dissolution medium on the in vitro release of the drug was studied.

Kayitare E[47] *et al.,* Designed paediatric antiretroviral formulations on the market, a novel fixed dose combination (FDC) tablet containing 300mg zidovudine (AZT) and 150mg lamivudine (3TC) was developed to improve dosing accuracy and allow flexible drug dosing in function of the body weight of paediatric HIV patients as recommended by WHO.

Talukdar[48] *et al.,* Have done the comparative study on xanthan gum and HPMC as matrices for controlled release drug delivery. They have concluded that drug diffusion in hydrated HPMC matrices is higher than in hydrated xanthan gum matrices. This showed that xanthan gum has higher ability than HPMC to retard the release of drug when used as matrix forming agent.

Etentakis[49] *et al.,* Developed and evaluated the oral multiple unit and single unit Hydrophilic controlled release systems. Single unit dosage forms i.e. tablet and capsule and multiple unit dosage form i.e. mini tablets in capsule were prepared. Tablets and mini tablets showed greater sustained release effect compared with capsules.

Kranz[50] *et al.,* Studied the drug release mechanism from HPMC matrices and developed a new model for quantitative predictions of controlled drug delivery.

Katikaneni and Neau[51] ***et al.,*** Used ethyl cellulose for formation of matrix controlled release tablets of a water soluble drug. They have prepared tablets with direct compression technique. They concluded that ethyl cellulose matrices showed sustained drug release over a period of 10 h. and the lower viscosity grades of ethyl cellulose are more compressible than higher viscosity grades resulting in harder tablets and slower release rates.

Ford and Velasco[52] ***et al.,*** Studied the influence of drug: (HPMC) (Hydroxymethylpropylcellulose) ratio and other technological factors such as drug and polymer particle size and compression force on the drug release from the matrices of HPMC. The influence was assessed by multiway analysis of variance. They reported that release from HPMC ratio. The particle size also influenced the release to lesser extent and the compression force didn't affect the release parameters.

Divya A[53] ***et al.,*** **Bilayer tablet technology,** Bilayer tablet is new era for the successful development of controlled release formulation along with various features to provide a way of successful drug delivery system.

Sachin S. Kale[54] ***et al.,*** Bilayer tablet is improved beneficial technology to overcome the shortcoming of the single layered tablet. There is various application of the bilayer tablet it consist of monolithic partially coated or multi-layered matrices. In the case of bilayered tablets drug release can be rendered almost unidirectional if the drug can be incorporated in the upper nonadhesive layer its delivery occurs into the whole oral cavity.

Krishna Vamshi Allam[55] ***et al.,*** This review briefly discusses about the novel dosage forms like controlled release matrix tablets, floating tablets, nanoparticles, microparticles, liposomes, and niosomes; which may possibly suitable for the controlled and/or sustained release of Lamivudine and thus, useful in developing the more effective AIDS therapy with very less or no adverse side effects.

Remeth Jacky Dias[56] ***et al.,*** This study was to design and optimize an oral controlled release acyclovir mucoadhesive tablet, in term of its drug release and mucoadhesive strength. A 32 full factorial design was employed to study the effect of independent variables like Carbopol-934P and Hydroxypropyle methylecellulose K100, wchich significantly influence charactarastics like swelling index, ex-vivo mucoadhesive strength and in-vitro drug release.

MA Naeem[57] ***et al.,*** Developed and characterize bilayer tablet formulations of tramadol HCl (TmH) and acetaminophen (AAP) microparticles.

P.Hiremath[58] ***et al.,*** Studied the design and controlled release matrix tablets of Rifampicin and Isoniazid using HPMC.

S.B. Tiwari[59] ***et al.,*** Studied the influence of hydrodynamic conditions on Verapamil hydrochloride release from a hydrophilic matrix using ionic and non-ionic polymers.

Kiattisalc Saeia[60] ***et al.,*** Studied the factors influencing drug dissolution characteristic from hydrophilic polymer matrix tablet.

Chapter III

Aim and Objective

3. AIM AND OBJECTIVES

AIM OF THE STUDY:

Lamivudine and Zidovudine are belongs to the class of Nucleoside reverse transcriptase inhibitor which are most widely used in the treatment of HIV infection either alone or in combination with other antiviral drugs to increase efficacy, minimize resistance and side effect. The advantage of the development of controlled release dosage form is reducing the frequent dosing and improves the patient compliance.

The main aim of this work was to formulate and *in vitro* evaluate controlled release bilayer matrix tablet of Lamivudine and Zidovudine by using hydrophilic polymer HPMC (K 100 and K 15) of different grades and different concentration to study the drug release pattern.

OBJECTIVE OF THE STUDY:

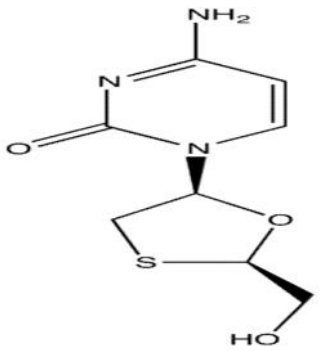
- To formulate the Oral controlled release bilayer matrix tablet of Lamivudine and Zidovudine.
- To study the effect of different grades of polymer (HPMC K 100 and HPMC K 15) on drug release .
- Evaluation of the post-compression parameters and drug release profile.
- Selection of the best formulation.
- To analyse the mechanism of drug release for best formulation.
- To avoid the side effect of the Drug.
- To improve the patient compliance.
- To improve the antiviral activity.
- To reduce the risk of emergence of resistance to antiretroviral drugs.

Chapter IV

Drug Profile

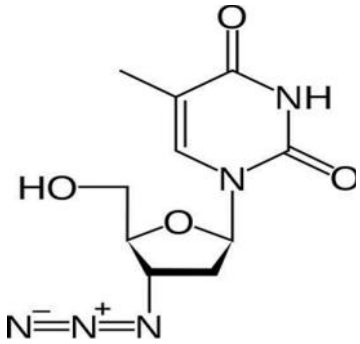
4. DRUG PROFILE

Table No 4.1. Drug Profile of Lamivudine[84,85&86]:

SR. NO.	PARTICULARS	DESCRIPTION
1	**Name**	Lamivudine
2	**Category**	Anti-Retroviral agent
3	**Chemical Formula**	$C_3H_{11}N_3O_3S$
4	**Molecular Weight**	**229.2560**
5	**IUPAC Name**	4-amino-1-[(2R,5S)-2-(hydroxyl methyle)-1,3-oxothioline-5-yl] pyramidine-2-one.
6	**Chemical Structure**	
7	**Half Life**	5-7 hrs.
8	**Dosage Form**	Tablet, Capsule etc.
9	**Solubility (mg/ml)**	70 mg/ml(water)
10	**Max. Daily Dose**	300 mg/day
11.	**Melting pt.**	160-162^{0}C

12	**Protein Binding**	<36%
13	**Bioavailability**	80-87%
14	**Absorption**	* After oral administration it is rapidly absorb through gastrointestinal tract.
15	**Distribution**	* Believed to be distributed into the extravascular spaces. * Vd is independent of dose and doesn't correlate with body weight less than 36% is bound to plasma proteins.
16	**Metabolism**	* Metabolism is the minor route of elimination. The only known metabolite is trans sulfoxide metabolite.
17	**Excretion**	Primarily eliminated unchanged in urine.
18	**Mechanism Of Action**	* Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) with active against Human Immunodeficiency Virus Type-1 (HIV-1) and Hipatitis b (HBV). * Lamivudine is phosphorylated to active metabolite that compete for incorporation into viral DNA. * They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. Thus the viral DNA growth is Terminated.
19	**Indication**	Lamivudine are a nucleoside analogue indicated in combination with other antiretroviral agents for the treatment of human immunodeficiency virus (HIV-1) infection. Limitation of use: The dosage of this product is for HIV-1 and not for HBV.

Table No. 4.2. Drug Profile of Zidovudine[84,85&87]:

Sr. No.	PARTICULARS	DESCRIPTION
1	**Name**	Zidovudine.
2	**Category**	Anti-Retroviral agent
3	**Chemical Formula**	$C_{10}H_{13}N_5O_4$
4	**Molecular Weight**	267.2413
5	**IUPAC Name**	1-[(2R,4S,5S)-4-azido-5(Hydroxy-methyl)oxolan-2-yl]-5-methyl-1,2,3,4,-tetra hydroxy pyrimidine-2-,4dione.
6	**Chemical Structure**	 ZIDOVUDINE
7	**Half Life**	0.5-3 hours
8	**Dosage Form**	Tablet, Capsule etc.
9	**Max.Daily dose**	600mg/day
10	**Solubility (mg/ml)**	10-50 gm/L at 17^0C Soluble in water.
11	**pKa**	9.96
12	**Melting pt.**	106-112^0C
13	**Protein Binding**	**30-38%**
14	**Bioavailability**	**52-75%**
15	**Absorption**	After oral administration it is rapidly absorb through gastrointestinal tract.

16	**Distribution**	Zidovudine is extensively distributed. Binding to plasma protein is low, less than 38%. Apparent Vd is 1.6 L/kg.
17	**Metabolism**	Hepatic metabolism. Major metabolites are 3′-azido-3′-deoxy-5′-O-beta-D-glucopyranuronosylthymidine (GZDV) and 3′-amino-3′-deoxythymidine (AMT).
18	**Excretion**	Primarily eliminated by hepatic metabolism. Elimination half-life is 0.5 to 3 h. GZDV (74%) and zidovudine (14%) are recovered in the urine. Renal Cl is 0.34 l/h/kg.
19	**Mechanism Of Action**	❖ It is a reverse transcriptase inhibitor. ❖ The drug enters the host cell by diffusion and is phosphorylated by cellular thymidine kinase. ❖ It then converts monophosphate into di and tri-phosphate. ❖ This zidovudine triphosphate competitively inhibit reverse transcriptase.
20	**Indication**	In combination with other antiretroviral agents for the treatment of HIV-1 infections; prevention of maternal-fetal HIV-1 transmission.

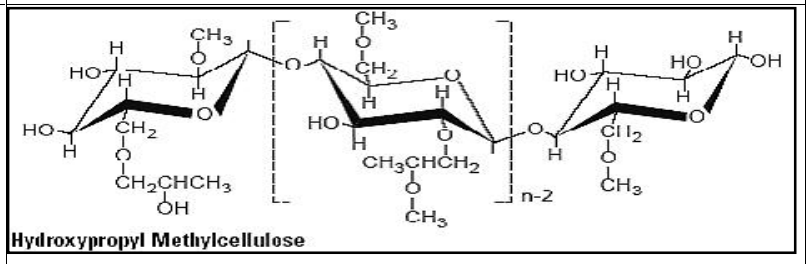
Chapter V

Polymer and Excipient Profile

5. POLYMER AND EXCIPIENT PROFILE

5.1. HYDROXYPROPYL METHYLCELLULOSE[70,71,73&80]:

Table No. 5.1. Polymer Profile of Hydroxypropyl Methyl Cellulose.

Synonyms	Cellulose, hydroxypropyl methyl ether, HPMC, methocel, metolose
Chemical Formula	HPMC is a partially o-methylated and o-(2-hydroxypropylated) cellulose. It is available in several grades, vary in viscosity and extent of substitution.
Structural Formula	 Hydroxypropyl Methylcellulose
Solubility	Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol, and ether, but soluble in mixture of ethanol and dichloromethane, and mixture of methanol and dichloromethane.
Molecular Weight	100 – 150000
Description	HPMC is an odorless and tasteless, white or creamy white colored fibrous or granular powder.
Functional Category	Coating agent, film-former, stabilizing agent, suspending agent, tablet binder, and viscosity increasing agent.
Stability and Storage Conditions	HPMC is a stable material although it is hygroscopic after drying. Solutions are stable between pH 3-11. Increasing temperature reduces the viscosity of solutions. It undergoes a reversible sol to gel transformation upon heating and cooling respectively. Stored in a well closed container, in a cool, dry place.
Incompatibilities	It is incompatible with some oxidizing agent. Since it is

	nonionic, it will not complex with metallic salts and ionic organics to form insoluble precipitates.
Safety	It is generally regarded as a nontoxic and non-irritant material although excessive oral consumption may have a laxative effect.
Applications	HPMC is widely used in oral and topical pharmaceutical formulations. In oral products it is primarily used as a tablet binder, film coating and as an extended release tablet matrix. Concentration between 2-5% w/w may be used as a binder either in wet or in dry granulation process. High viscosity grades may be used to retard the release of water soluble drugs from a matrix. Lower viscosity grades are used in aqueous film coating while higher viscosity grades are used with organic solvents.

5.2. MICROCRYSTALLINE CELLULOSE[71,73&80]:

Table No. 5.2 Excipient Profile of Microcrystalline Cellulose.

Synonyms	Celex, cellulose gel, Celphere, Emocel, and Pharmacel.
Molecular Weight	36000
Functional Category	Tablet and capsule diluent.
Description	Microcrystalline cellulose is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades having different properties and applications.
Solubility	slightly soluble in 5% w/v sodium hydroxide solution but practically insoluble in water, dilute acids and most organic solvents.

Stability and storage conditions	It is stable, hygroscopic material The bulk material should be stored in a well closed container in a dry, cool, place.
Incompatibility	Incompatible with strong oxidizing agents.
Safety	It is widely used in oral pharmaceutical formulations and food products. It is generally regarded as a nontoxic and non-irritant material.
Applications	Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder / diluent in oral tablet and capsule formulation. It is used in both wet granulation and direct compression processes. In addition to its use as binder / diluent, also be used as lubricant and disintegrant agent in tableting.

5.3. POLY VINYL PYROLIDONE (PVP)[71,73&80]:

Table No. 5.3. Polymer Profile of Polyvinyl Pyrrolidone.

Synonyms	Povidone, polyvinylpyrrolidone, PVP, kollidone, and plasdone.
Chemical formula	1-ethenyl-2-pyrrolidinone homopolymer. $(C_6H_9NO)_n$
Molecular weight	2500-3000000.
Description	It is white to creamy white, odorless or almost odorless, hygroscopic powder
Functional category	Tablet binder, suspending or viscosity increasing agent
Solubility	Readily soluble in water, organic solvents including monohydric (ethanol, methanol) and polyhydric alcohols, acids, esters, ketones, and chloroform

Stability and storage conditions	Aqueous solutions are susceptible to growth of molds and consequently required the addition of suitable preservatives.
Incompatibility	It forms molecular adducts in solution with sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin, and other compounds
Safety	Chemically, PVP is inert and nontoxic. It does not irritate the mucous membrane of rabbit eyes, antigenic property and does not interfere in antibody formation studies.
Applications	Carrier for drug, dispensing agent, suspending or viscosity builder, tablet binder, tablet diluents and coating agent.

5.4. MAGNESIUM STEARATE[71,73&80]:

Table No. 5.4. Excipient Profile of Magnesium Stearate.

Synonyms:	Magnesium octadecanoate, octadecanoic acid, magnesium salt, stearic acid, and magnesium salt.
Chemical Formula:	$C_{36}H_{70}MgO_{4.}$
Molecular weight	591.34
Functional Category	Tablet and capsule lubricant.
Description	Magnesium stearate is a fine, white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. The powder is greasy to the touch and readily adheres to the skin.
Solubility	Practically insoluble in ethanol, ether, and water, slightly soluble in warm benzene and warm ethanol.

Stability and storage conditions	Magnesium stearate is stable and should be stored in a well closed container in a cool, dry place**.**
Incompatibilities	Incompatible with strong acids, alkalis, and iron salts. Avoid mixing with strong oxidizing materials. Magnesium stearate cannot be used in products containing aspirin, some vitamins, and most alkaloidal salt.
Applications	Magnesium stearate is widely used in cosmetics, foods, and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25% and 5.0% w/w. It is also used in barrier creams.

5.5. COLLOIDAL SILICON DIOXIDE[71,73&80]:

Table No. 5.5. Excipient Profile of COLLOIDAL SILICON DIOXIDE .

Synonyms	Aerosil, colloidal anhydrous silica, anhydrous silicic acid, silicic anhydride, silicon dioxide fumed.
Chemical formula	SiO_2
Description	Light, loose, bluish-white coloured, odorless, tasteless, nongritty amorphous powder
Functional category	Adsorbent, anticacking agent, emulsion stabilizer, glidant, Suspending agent, tablet disintegrant, thermal stabilizer, Viscosity increasing agent.
Solubility	Practically insoluble in organic solvents, water and acids. Soluble in hot solutions of alkali hydroxide.
Stability and storage	It should be stored in a well-closed container.
Safety	It should not be administered parenterally
Applications	It is used as glidant in tabletting, to stabilize emulsions and as a thixotropic thickening agent, suspending agent in gels and semisolid preparations, to promote particulate suspension in aerosols. It is also used tablet disintegrant and adsorbent dispersing agent for liquids in powders. It is frequently added to suppository formulations containing lipophilic excipients to increase viscosity, prevent sedimentation during molding, and decrease the release rate.

Chapter VI

Plan of Work

6. PLAN OF WORK

PHASE - I

- **Preformulation studies**

- Preparation of calibration curve**.**
- Drug characterisation
- Drug Excipients compatibility Studies(FTIR)
- Bulk density
- Tapped density
- Compressibility index
- Angle of repose
- Hausner's ratio

PHASE – II

- **Preparation of bilayer tablets by direct compression method.**

PHASE – III

- **Evaluation of prepared tablets**
    - Appearance
    - Hardness
    - Weight variation test
    - Friability
    - Thickness
    - Assay
    - Dissolution Study by using Phosphate Buffer pH 6.8.
    - Kinetic study for the best formulation.
    - Stability studies as per ICH guideline for the best formulation.

Chapter VII

Materials and Equipment's

7. MATERIALS AND EQUIPMENTS

7.1. MATERIAL (DRUG, POLYMER AND CHEMICALS):

Table No 6.1. Name of the Materials (Drug, Polymers and chemicals) and suppliers.

Sr. No.	Name of the Ingredients	Name of the Suppliers
1	Lamivudine (API)	Lupin Research Park, Pune.
2	Zidovudine (API)	Wockhardt Pharmaceuticals Pvt. Ltd, Aurangabad.
3	Hydroxypropylmethylcellulose K 100 M	Themis Research lab, Mumbai
4	Hydroxypropylmethylcellulose K 15 M	Themis Research lab, Mumbai
5	Aerosil	Themis Research lab, Mumbai
6	Magnesium stearate	Research lab, Mumbai.
7	Microcrystalline Cellulose	Themis Research lab, Mumbai
8	Polyvinyl Pyrrolidone (K-30)	Research lab, Mumbai.
9	Red oxide of Iron	Themis Research lab, Mumbai
10	Methanol	Sisco research lab, Mumbai
11	Potassium dihydrogen phosphate	Research lab, Mumbai.
12	Sodium hydroxide	Research lab, Mumbai.
13	Potassium bromide	Research lab, Mumbai.
14	Hydrochloric Acid	Research lab, Mumbai.
15	Potassium Chloride	Research lab, Mumbai.

6.2. EQUIPMENTS:

Table No. 6.2. Name of Equipment and Manufacturer.

Sr. No.	Name of Equipments	Name of the Manufacturer
1	Rotary tablet compression machine	Karnavati engineers pvt.ltd, Mumbai (Model: MINI PRESS-II MT).
2	USP Tablet Dissolution Apparatus (Type II)	Electrolab (Model:TDT-06P, Mumbai).
3	HPLC	Waters 2965, Shimadzu, Japan.
4	Hardness Tester	Pfizer, Inlap ltd, Mumbai
5	Electronic balance	Shimatzu, Japan (Model: AUY220).
6	Roche friability tester	Lab India, Mumbai, (Model: FT1020).
7	pH meter	Lab India, Mumbai, (Model: GMPH).
8	UV- Spectrophotometer.	Jasco, Japan (Model: V-530 & V-630).
9	Rotary Shaker	Remi instruments, vasai.
10	FT-IR spectrophotometer	Shimadzu (Model: FTIR-8400S), Japan.
11	Tap density tester	Lab India, Mumbai.(Model:TD1025).
12	Melting Point apparatus	VEEGO, Mumbai. (Model: VMP-D).

Chapter VIII

Methodology

8. METHODOLOGY

8.1.1 Preparation of 0.2 M potassium dihydrogen phosphate[62]:

0.2 M potassium dihydrogen phosphate was prepared according to IP 1996. A quantity of 27.2 gm of potassium dihydrogen phosphate was dissolved in water and make up the volume to 1000 ml using water.

8.1.2 Preparation of 0.2 M NaOH[62]:

0.2 M NaOH prepared by dissolving 8 gm of NaOH in to water and make up the volume to 1000 ml using water.

8.1.3 Preparation of phosphate buffer pH 6.8[62]:

The phosphate buffer pH 6.4 prepared according to IP 1996 by mixing the 50 ml of 0.2 M potassium dihydrogen phosphate and 39.1 ml of 0.2 M NaOH and make up the volume to 200 ml using water.

Calibrations curve of Lamivudine in Phosphate buffer pH 6.8:

8.1.4 Procedure[62]:

An accurately weighed required quantity of Lamivudine was dissolved in phosphate buffer pH 6.8 to obtain a solution having known concentration of 5, 10, 15, 20 and 25 µg/ml.. Then absorbance of Lamivudine was determined at 270 nm. The calibration curve is shown in **Figure No 9.1** and absorbance of different concentration of Lamivudine is shown in **Table No 9.1.**

8.1.5 Scanning of Lamivudine in phosphate buffer pH 6.8[62]:

The absorption maxima of the standard solution were scanned between 200-400 nm on JASCO UV V-630 spectrophotometer. The absorption maxima were found to 270 nm.

8.2 Drug characteristics[62] :

8.2.1 Appearance, colour:

The sample obtained was examined for its appearance and colour.

8.2.2 Solubility:

The solubility of obtained compound was determined in acetone, methylene chloride, methanol, alkali hydroxide and water.

8.2.3 Identification test:

About 2 mg of drug was dissolve in methanol and diluted to 100 ml with the same solvent and examined between 220 nm- 350nm, the solution shows two absorbance maxima at 226 nm & 270 nm. The ratio of the absorbance was measured at maxima 226 nm to that 270 nm; it was must be in between 2.0 to 2.4.

8.2.4 Assay[62]:

Assay or percentage purity of Lamivudine is done by UV method.

8.2.5 Melting point[63]:

The sample obtained was characterized for melting point of the substance. The melting point was determined by introducing amount of substance in the capillary attached to graduated thermometer and constant heat was applied with the assembly suspended in the paraffin bath. The drug sample was tested in temperature range 100-200^{0}c and teat which drug melts was noted.

8.2.6 UV-Visible spectroscopy[63]:

The Lamivudine was dissolved in methanol and resultant solution was scanned between 200-400 nm to determine its absorption maxima**.**

8.2.7 FT-IR spectroscopy[63&69]:

FT-IR spectrum of drug sample was recorded as potassium bromide (KBr) pellets at resolution of 4cm^{-1} for its authentication and to study principle peaks using Jasco FT-IR (JAPAN) spectrophotometer. The identified peaks were compared with the principle peaks of reported IR spectrum the sample was authenticated.

8.3 Drug – Excipients Compatibility Studies[63&69]:

These studies were performed in order to confirm the drug- exciepient compatibility. These studies mainly include FTIR given below,

8.3.1 FT-IR spectroscopy study[63&69]:

FT-IR spectra of pure drug, Pure HPMC K100 M & K15 of this polymer with drug were recorded on Jasco FT-IR spectrophotometer using KBr discs. The instrument was operated under dry air purge and the scans were collected at scanning speed 2 mm/sec with resolution of 4 cm^{-1} over the region 4000-400 cm^{-1}. The scans were evaluated for presence of principle peaks of drug, shifting and masking of drug peaks and appearance of new peaks due to polymer interaction. The FT-IR spectra of pure Lamivudine, Pure HPMC K100 M, HPMC K15 M and physical mixture are shown in **Figure No 9.2, 9.3, 9.4 and 9.5.**

8.4 Evaluation of powder properties[2,64&67]:

The powder properties include Bulk density, Tap density, Hausner ratio, Carr's index were determined using tap density tester (Lab India).

8.4.1 Bulk Density:

The bulk density, as a measure used to describe packing materials or granules, was determined by transferring the accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. Ratio of weight of the sample to the volume it occupied was calculated.

The bulk density is obtained by dividing the weight of the sample in grams by final volume1 in cm^3.

$$\mathbf{b = M / V_p} \quad \text{……..……(8.1)}$$

Where, b = bulk density, M = weight of sample in grams,

V_p = final volumes of Powder in cm^3.

8.4.2 Tap Density:

Weighed powder sample was transferred to a graduated cylinder and was placed on the tapped density test apparatus, was operated for a fixed number of taps (100). The tapped density was determined as the ratio of weight of sample to tapped volume.

The Tap density is obtained by dividing the weight of the sample in grams by final Tap volume in cm^3

$$\mathbf{T = M / V_T} \quad \text{……..…..…(8.2)}$$

Where,

T = Tap density

M = weight of sample in grams

V_T = final Tap volumes of Powder in cm^3.

4.4.3 Angle of repose (¯):

A funnel was fixed at a particular height on a burette stand. A graph paper was placed below the funnel on the table. The powdered drug passed through the funnel until it forms a pile. The radius of the pile was noted down. Angle of repose of the powder material was calculated using the formula.

The frictional forces in a loose powder can be measured by the angle of repose(θ). This is the maximum angle possible between the surface of a pile of powder and the horizontal plane and it is given as,

$$\text{Tan } \theta = h / r$$

$$\theta = \tan^{-1}[h/r] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8.3)$$

Where,

θ =the angle of repose

h = the height in cm

r = the radius.

The powder mixture was allowed to flow through the funnel fixed to a stand at definite height. The angle of repose was then calculated by measuring the height and radius of the heap of powder formed.

Table No.8.1 : Angle of Repose

Sr. No.	Flowability	Angle of Repose
1	Excellent	$25 - 30^0$
2	Good	$30 - 35^0$
3	Fair	$35 - 37^0$
4	Poor	$37 - 45^0$
5	Very poor	Above 45^0

8.4.4Carr's index:

The Carr's index is determined from the tapped density and poured density (bulk density) as per the formula given below.

Carr's index (%) = Tapped density- poured density

---------- X 100…… (8.4)

Tapped density

Table No.8.2:Carr's index

Carr's index %	Type of flow
5 - 15	Excellent
12 – 18	Good
18 – 23	Fair to passable
23 – 35	Poor
35 – 38	Very poor
>40	Extremely poor

8.4.5 Hausner ratio:

Hausner ratio is determined from the ratio of tapped density to poured density using formula given below,

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Poured density}} \quad \text{……..} \quad (8.5)$$

B] FOR ZIDOVUDINE

8.1.1. Calibrations curve of Zidovudine in Phosphate buffer pH 6.8[62]:

8.1.2.5 Procedure:

An accurately weighed quantity of Zidovudine was dissolved in phosphate buffer pH 6.8 to obtain a solution having known concentration of 5, 10, 15, 20 and 25 µg/ml. Then absorbance of Zidovudine was determined at 267 nm. The calibration curve is shown in **Figure No. 9.6** and absorbance of different concentration of Zidovudine is shown in **Table No. 9.4.**

8.1.1.4 Scanning of Zidovudine in phosphate buffer pH 6.8[63&69]:

The absorption maxima of the standard solution were scanned between 200-400 nm on JASCO UV V-630 spectrophotometer. The absorption maxima were found to 267 nm.

8.2 Drug characteristics[61,67&72] :

8.2.1 Appearance, colour:

The sample obtained was examined for its appearance and colour.

8.2.2 Solubility:

The solubility of obtained compound was determined in acetone, methylene chloride, methanol, alkali hydroxide and water.

8.2.3 Identification test[63]:

About 2 mg of drug was dissolve in methanol and diluted to 100 ml with the same solvent and examined between 220 nm- 350 nm, the solution shows two absorbance maxima at 226 nm & 267 nm. The ratio of the absorbance was measured at maxima 226 nm to that 267 nm; it was must be in between 2.0 to 2.4.

8.2.4 Assay[61]:

Assay or percentage purity of Zidovudine is done by UV method.

8.2.5 Melting point[63]:

The sample obtained was characterized for melting point of the substance. The melting point was determined by introducing amount of substance in the capillary attached to graduated thermometer and constant heat was applied with the assembly suspended in the paraffin bath. The drug sample was tested in temperature range 100-200^{0}c and teat which drug melts was noted.

8.2.6 UV-Visible spectroscopy[63&69]:

The Zidovudine was dissolved in methanol and resultant solution was scanned between 200-400 nm to determine its absorption maxima.

8.3 Drug – Excipients Compatibility Studies[63&69]:

These studies were performed in order to confirm the drug- exciepient compatibility. These studies mainly include FTIR given below,

8.3.1 FT-IR spectroscopy study[63&69]:

FT-IR spectra of pure drug, Pure HPMC K100 M & physical mixtures of this polymer with drug were recorded on Jasco FT-IR spectrophotometer using KBr discs. The instrument was operated under dry air purge and the scans were collected at scanning speed 2 mm/sec with resolution of 4 cm^{-1} over the region 4000-400 cm^{-1}. The scans were evaluated for presence of principle peaks of drug, shifting and masking of drug peaks and appearance of new peaks due to polymer interaction. The FT-IR spectra of pure Zidovudine, Pure HPMC K100 M, HPMC K15 M and physical mixtures are shown in **Figure No 9.7, 9.8, 9.9 and 9.10.**

8.4 Evaluation of powder properties[62,64,72&74]:

The powder properties include Bulk density, Tap density, Hausner ratio, Carr's index were determined using tap density tester (Lab India).

8.4.1 Bulk Density:

The bulk density, as a measure used to describe packing materials or granules, was determined by transferring the accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. Ratio of weight of the sample to the volume it occupied was calculated.

The bulk density is obtained by dividing the weight of the sample in grams by final volume1 in cm^3.

$$B = M / V_p \quad \text{……..……(8.6)}$$

Where, B = bulk density M = weight of sample in grams

V_p = final volumes of Powder in cm^3.

8.4.2 Tap Density:

Weighed powder sample was transferred to a graduated cylinder and was placed on the tapped density test apparatus, was operated for a fixed number of taps (100). The tapped density was determined as the ratio of weight of sample to tapped volume.

The Tap density is obtained by dividing the weight of the sample in grams by final Tap volume in cm^3

$$T = M / V_T \quad \text{…….…..…(8.7)}$$

Where,

T = Tap density

M = weight of sample in grams

V_T = final Tap volumes of Powder in cm^3.

8.4.3 Angle of repose (¯):

A funnel was fixed at a particular height on a burette stand. A graph paper was placed below the funnel on the table. The powdered drug passed through the funnel until it forms a pile. The radius of the pile was noted down. Angle of repose of the powder material was calculated using the formula.

The frictional forces in a loose powder can be measured by the angle of repose θ. This is the maximum angle possible between the surface of a pile of powder and the horizontal plane and it is given as,

Tan θ = h / r,

$\theta = \tan^{-1}[h/r]$…… (8.8)

Where,

θ = the angle of repose

h = the height in cm

r = the radius.

The powder mixture was allowed to flow through the funnel fixed to a stand at definite height. The angle of repose was then calculated by measuring the height and radius of the heap of powder formed.

Table No.8.3: Angle of Repose

Sr. No.	Flowability	Angle of Repose
1	Excellent	$25 - 30^0$
2	Good	$30 - 35^0$
3	Fair	$35 - 37^0$
4	Poor	$37 - 45^0$
5	Very poor	Above 45^0

8.4.4 Carr's index:

The Carr's index is determined from the tapped density and poured density (bulk density) as per the formula given below,

$$\text{Carr's index (\%)} = \frac{\text{Tapped density - poured density}}{\text{Tapped density}} \times 100 \ldots\ldots\ldots\ldots (8.9)$$

Table No.8.4: Carr's index

Carr's index %	Type of flow
5 - 15	Excellent
12 – 18	Good
18 – 23	Fair to passable
23 – 35	Poor
35 – 38	Very poor
>40	Extremely poor

8.4.5 Hausner ratio:

Hausner ratio is determined from the ratio of tapped density to poured density using formula given below.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Poured density}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (8.10)$$

8.5 Preparation of Lamivudine And Zidovudine Controlled release Bilayer Tablets[53&54]:

The tablets were prepared by direct compression technique. Before blending of drug and other Excipients, they were sifted through sieve no. 40 to remove any large particles. Drugs and other Excipients were blended for 10 mins. Then, subsequently this powder mixture was blended for 5 mins. with magnesium stearate. This mixture was directly compressed to get the tablets.

a) Preparation of Lamivudine controlled release layer:

DOSE CALCULATION (Theoretical Release Profile)

Total drug of Lamivudine for controlled release formulation was calculated by the following equation using available pharmacokinetic data..

$$Dt = Dose(1+0.639Xt/t_{1/2})$$

Where, Dt=Total dose of Drug, Dose= Dose of immediate release part (50mg), t= time (hours) during which the controlled release is desired (12hours), $t_{1/2}$= half-life of the drug(7 Hours).

$$Dt=50(1+0.639X12/7)=140.5 \text{ mg}$$

Hence, Lamivudine active 150mg taken as controlled release per tablet.

Table No.8.5: Lamivudine layer formulation.

Sr. No.	Ingredients (mg)	LF1	LF2	LF3	LF4	LF5	LF6	LF7	LF8	LF9
1	Lamivudine	150	150	150	150	150	150	150	150	150
2	HPMC (K100)	15	18.75	22.5	_	_	_	7.5	9.37	11.25
3	HPMC(K15)	_	_	_	30	37.5	45	15	18.75	22.5
4	MCC	15	15	15	15	15	15	15	15	15
5	PVP	15	15	15	15	15	15	15	15	15
6	Aerosil	5	5	5	5	5	5	5	5	5
7	Mg.Stearate	5	5	5	5	5	5	5	5	5
8	Red oxide iron	q.s	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
	Total wt.	205	208.75	212.5	220	227.5	235	212.5	218.12	223.75

The Lamivudine controlled release layer containing uniform mixture of Lamivudine and all excipients are sifted through 40# sieve and mixed well for10minutes. And then lubricated with magnesium stearate (presifted through 40#), the well-mixed powder blend were used as upper layer.

b) Preparation of Zidovudine controlled release layer.

DOSE CALCULATION (Theoretical Release Profile)

Total drug of Zidovudine for controlled release formulation was calculated by the following equation using available pharmacokinetic data..

$$Dt = Dose(1+0.639Xt/t_{1/2})$$

Where, Dt=Total dose of Drug, Dose= Dose of immediate release part (100mg), t= time(hours) during which the controlled release is desired (12hours), $t_{1/2}$= half-life of the drug (3Hours).

$$Dt=100(1+0.639X12/3)= 307 \text{ mg.}$$

Hence, Zidovudine active 300 mg taken as controlled release per tablet.

Table No. 8.6: Zidovudine Layer formulations

Sr. No.	Ingredients (mg)	ZF1	ZF2	ZF3	ZF4	ZF5	ZF6	ZF7	ZF8	ZF9
1	Zidovudine	300	300	300	300	300	300	300	300	300
2	HPMC (K 100)	30	37.5	45	_	_	_	15	19	22.5
3	HPMC (K 15)	_	_	_	60	75	90	30	37.5	45
4	MCC	20	20	20	20	20	20	20	20	20
5	PVP	20	20	20	20	20	20	20	20	20
6	Aerosil	6	6	6	6	6	6	6	6	6
7	Mg. Stearate	6	6	6	6	6	6	6	6	6
	Total wt.	382	389.5	397	412	427	442	397	408.5	419.5

The controlled release layer containing uniform mixture of drug, polymers and excipients was mixed properly with weighed amount of polymers and Excipients. The well-mixed powder was compressed by direct Compression technique and is used as lower controlled release layer.

c) Preparation of bilayer tablets:

Bilayer tablets were prepared by combining of two formulations of controlled release layer. After the compression upper punch was lifted and the blend of powder first controlled release layer was poured into the die, containing initially compressed matrix tablet on RIMEK multi station punching machine.

8.6 Evaluation of Lamivudine and Zidovudine Controlled release Bilayer Matrix Tablet[2,62,68,72,74,77&83]:

Tablets prepared from powder blends were subjected to evaluation of properties including drug content uniformity, weight variation, tablet hardness, friability, thickness , in vitro drug release and kinetic study etc.

8.6.1 Weight Variation:

Procedure:

Twenty tablets were selected randomly and weighed by using Electronic balance (Shimatzu). Average weight of the tablet was determined. These tablets were weighed individually and the weight variation was determined.

Table No.8.7: Weight Variation Tolerance for Uncoated Tablets.

Sr. No.	Average Weight of Tablets (mg)	Maximum Percentage Difference Allowed (%)
1	130 or less	10
2	130 to 324	7.5
3	More than 324	5

8.6.2 Tablet hardness:

The resistance of tablets to shipping or breakage, under conditions of storage, transportation and handling before usage depends on its hardness. The hardness of tablet of each formulation was checked by using Pfizer hardness tester. The hardness was measured in terms Kg/cm^2.

8.6.3 Friability:

Friability was measure the tablet strength. Roche friabilator (Lab India) was used for testing the friability using the following procedure:

Twenty tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm dropping the tablets through a distance of six inches with each revolution. After 20min, the tablets were weighed and the percentage loss in tablet weight was determined.

8.6.4 Thickness:

Thickness of tablet is important for uniformity of tablet size. Thickness was measured by using Vernier Calipers. It was determined by checking ten tablets from each formulation.

8.6.5 *In vitro* Drug Release studies of Lamivudine and Zidovudine Controlled Release Matrix Bilayer Tablet[35,37,41&43]:

Tablets of all preliminary batches were subjected to dissolution rate studies. In-vitro dissolution studies were carried out on dissolution apparatus (ElectrolabModel: TDT-06P) to determine the drug release from various formulations. The 37 ± 0.5 °C Temperature maintain throughout the study. Studies were carried out in 900 ml of 6.8 phosphate buffer up to 12 hours at 100 rpm. Sample measuring 10 ml were withdrawn at 1, 2, 4,6,8,10,12 hr. and immediately replaced by equal volume of dissolution media equilibrated at the same temperature to maintain the volume. The sample withdrawn was passed through 0.45 µm. Then the resulting filtrate was evaluated by using HPLC. The row dissolution data

recorded was analyzed to calculate the amount of drug release and percentage cumulative drug release at different time intervals. The graphs of times vs. % cumulative release were plotted.

Details of dissolution test:

Dissolution test apparatus	:	USP TYPE II (DTD – 06P).
Speed	:	100 rpm
Stirrer	:	paddle type
Volume of medium	:	900 ml
Aliquot taken at each time interval	:	10 ml
Medium used	:	pH 6.8 Phosphate buffer
Temperature	:	37 ± 0.5 ^{0}C

8.6.6 Assay & Uniformity of content[41]:

8.6.6.1 Assay By HPLC:

Apparatus:

The HPLC system was waters HPLC consisted with the pump-alliance 2695 separation module, columnphenomenex luna 5µ C18 (12) 100A,(250×4.6×i.d,5µ), auto sampler, detector was waters 2996 and prominence diode array detector. Other instruments used were, Melter balance AY 220, Elico pH meter LI 127, Melter Ultrasonicatior and Millipore membrane filter. Shimadzu balance AY 220 and sonica ultrasonic cleaner were used.

Table No.8.8: Chromatographic Condition[41]:

Mobile Phase	**Acetonitrile: water (20:80)**
Column	Luna 5µ C18 (12) 100A,(250×4.6×i.d,5µ).
Flow rate	1.0 ml/min.
Detection(λ max)	270 nm (Isobestic pt.).
Column Oven	Ambient.
Injection Volume	20µL(loop injector).
Run Time	10 minutes.

Methods:

Preparation of Standard solutions:

Primary stock solution concentration of Lamivudine and Zidovudine 1000 µg/ml was prepared. All measurements were made at room temperature. The standard solutions were prepared by proper dilutions of the primary stock solution with acetonitrile and water (20:80) to obtain working standards in the concentration range of of Lamivudine and Zidovudine.

Sample preparation:

The tablet containing 300 mg Zidovudine and 150 mg Lamivudine were weighed, powdered and dissolved in acetonitrile and water (20:80) made to 1000 µg/ml. The stock solution of Zidovudine and Lamivudine was suitably diluted to give the mixture concentration of 30& 15, 45 & 30, 60 & 45, 75 & 60 and 90 &75 µg/ml for measurement. The contents were mixed thoroughly and filtered through 0.45µ membrane filter and sonicated for 20 min.

8.6.6.2 Uniformity of content[41]:

The drug content of ten dosage units was determined by HPLC method which is discussed above.

8.7 Data analysis [KINETIC STUDY] [7, 81&82]:

To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix and korsemeyer Peppas model using PSP-DISSO – v2 software. Based on the r-value, the best-fit model was selected.

Zero order kinetics:

Drug dissolution from pharmaceutical dosage forms that do not disaggregate and release the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation,

$$Q_t = Q_o + K_o t \quad \text{(8.11)}$$

Where,

Q_t = amount of drug dissolved in time t.

Q_o = initial amount of the drug in the solution and

K_o = zero order release constant.

First order kinetics:

To study the first order release rate kinetics, the release rate data were fitted to the following equation,

$$\log Q_t = \log Q_o + K_1 t/2.303 \quad \text{(8.12)}$$

Where,

Qt = the amount of drug released in time t,

Qo = the initial amount of drug in the solution,

K1 = the first order release constant.

Higuchi model[81]:

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semisolids and/or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. And the equation is,

Qt = KH ·t1/2……..…….. (8.13)

Where,

Qt = amount of drug released in time t,

KH = Higuchi dissolution constant.

Krosmeyer and Peppas release model[82]:

To study this model the release rate data are fitted to the following equation,

Mt / M ¥ = K · t n …… (8.14)

Where,

Mt / M ¥ = the fraction of drug release,

K = the release constant,

T = the release time,

N = the diffusional coefficient for the drug release that is dependent on the shape of the matrix dosage form.

Table No. 8.9 : Release Kinetics

Diffusion Exponent [Release exponent(n)]	**Overall solute diffusion mechanism (Drug transport mechanism)**
>0.45	Fickian diffusion
0.45<0.89	Anomalous (non-fickian) transport
0.89< 1	Case II
1<	Super case II

8.8 Stability studies for the best formulation[76] (As per ICH Guideline):

Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life." The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions, re-test periods and shelf-lives to be established.

ICH specifications for stability study:

Long term testing: 25 ^{0}C ± 2 ^{0}C /60% RH ± 5% RH for 12 months.

Accelerated testing: 40 ^{0}C ± 2 ^{0}C /75% RH ± 5% RH for 6 months.

Procedure:

In the present study, stability studies were carried out at 40^0 C and 75% RH for a specific time period up to 3 month for selected formulations. For stability study, the tablets were sealed in aluminum packaging coated inside with

polyethylene. These sample containers were placed in desiccator maintained at 75% RH.

NOTE: Saturated solution of sodium chloride at 40^0 C yields a 75% relative humidity.

Evaluation of samples:

The samples were analyzed for the following parameters:

I. Physical evaluation:

Appearance: The samples were checked for any change in colour at every week.

Hardness: The samples were tested for hardness at every week.

II. Chemical evaluation:

Drug content: The samples were checked for drug content.

Drug release: The samples were subjected to drug release studies.

Chapter IX

Results and Discussion

9. RESULT AND DISCUSSION

PREFORMULATION STUDIES:

A] FOR LAMIVUDINE:

Calibrations curve of Lamivudine in Phosphate buffer pH 6.8:

The calibration curve of the Lamivudine was prepared in the Phosphate buffer pH 6.8. Following **Table No. 9.1** shows the absorbance at λ_{max} 270 nm and **Figure No. 9.1** Shows the calibration curve with regression coefficient 0.9939, slope 0.0044 and the Y intercept +0.0048.

Table No.9.1: Data for Standard Curve of Lamivudine.

Sr. No.	Concentration (µg/ml)	Absorbance at 270nm
0	0	0
1	5	0.031
2	10	0.051
3	15	0.07
4	20	0.091
5	25	0.113

Calibration Curve of Lamivudine.

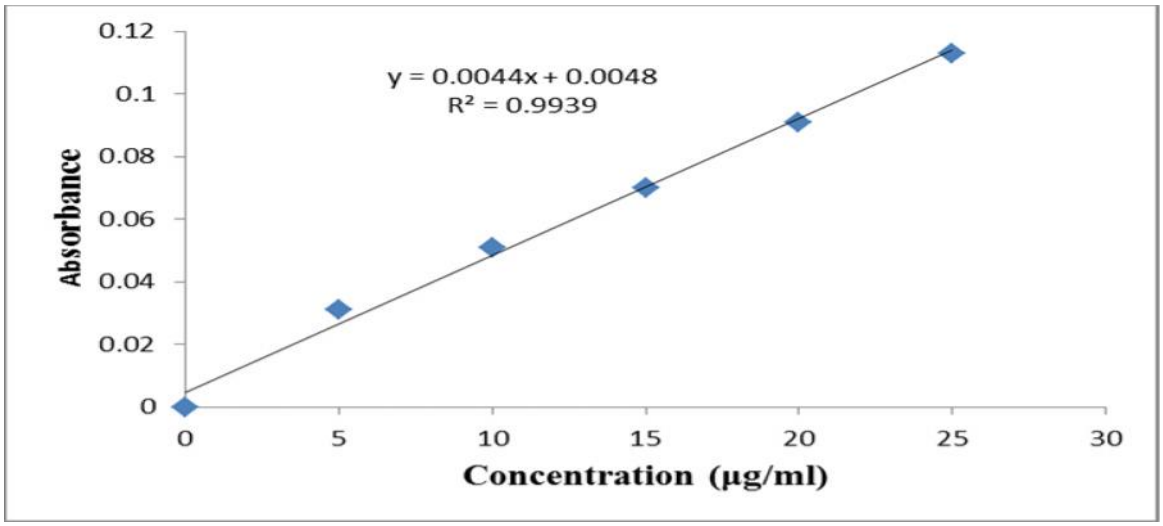


Figure No. 9.1: Calibration curve Graph of Lamivudine in pH 6.8 Phoshphate Buffer.

Physical Evaluation of Lamivudine

Appearance, colour:

The powder sample was found to be white and crystalline.

Solubility:

The Lamivudine powder was found to be freely soluble in water, methanol.

Identification Test:

The ratio of the absorbance measured at maxima 226nm to 270nm was found. That conform the sample taken was Lamivudine.

Assay:

The obtained sample of Lamivudine was found to be 99.17% pure by reported assay method.

Melting point:

The melting point was found to be 160-162^{0}C.

UV-Visible spectroscopy:

The Lamivudine was dissolved in methanol shows absorbance maximum at 270nm.

COMPATIBILITY STUDIES OF DRUG AND POLYMERS:-

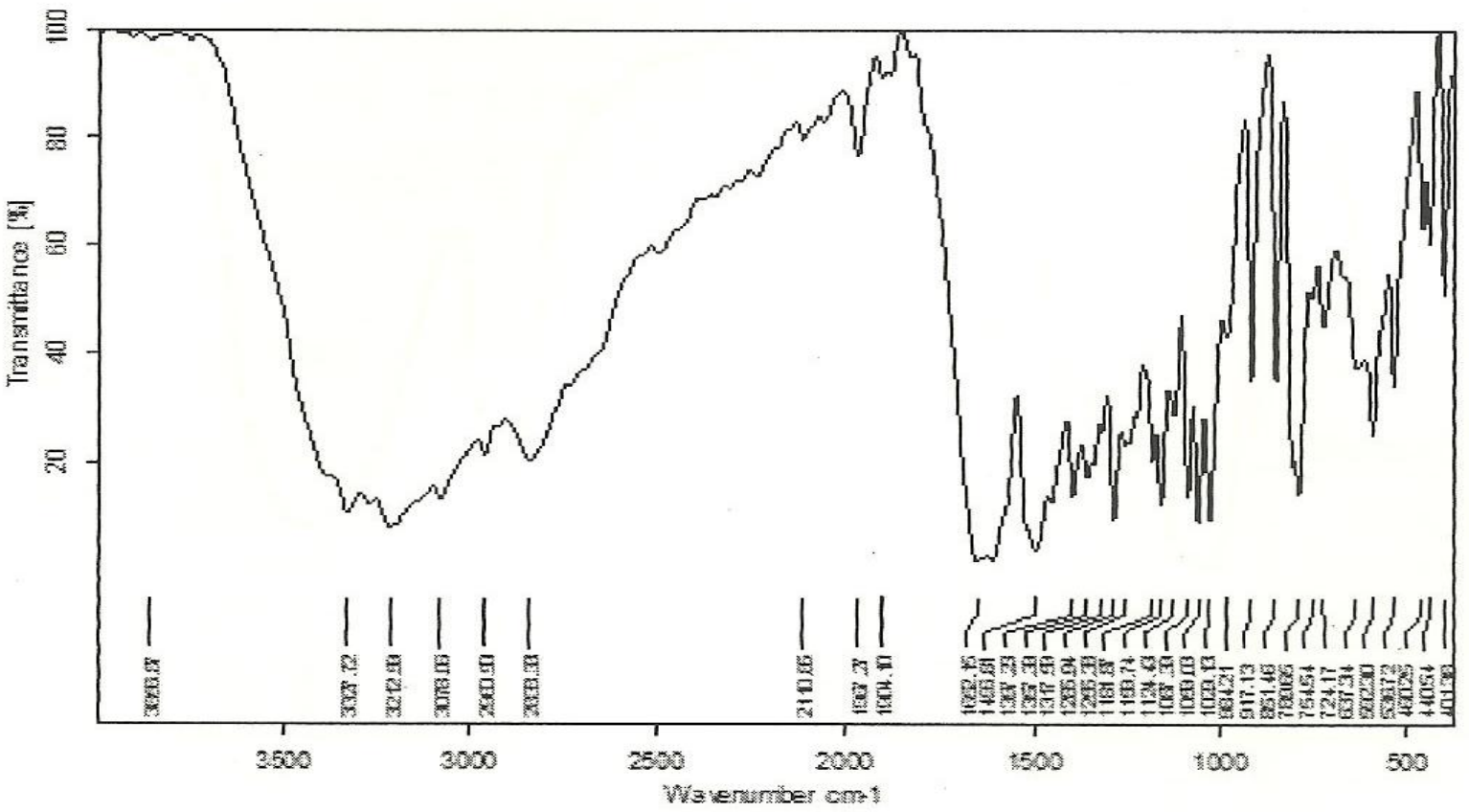


Figure No. 9.2: IR Spectrum of pure Lamivudine

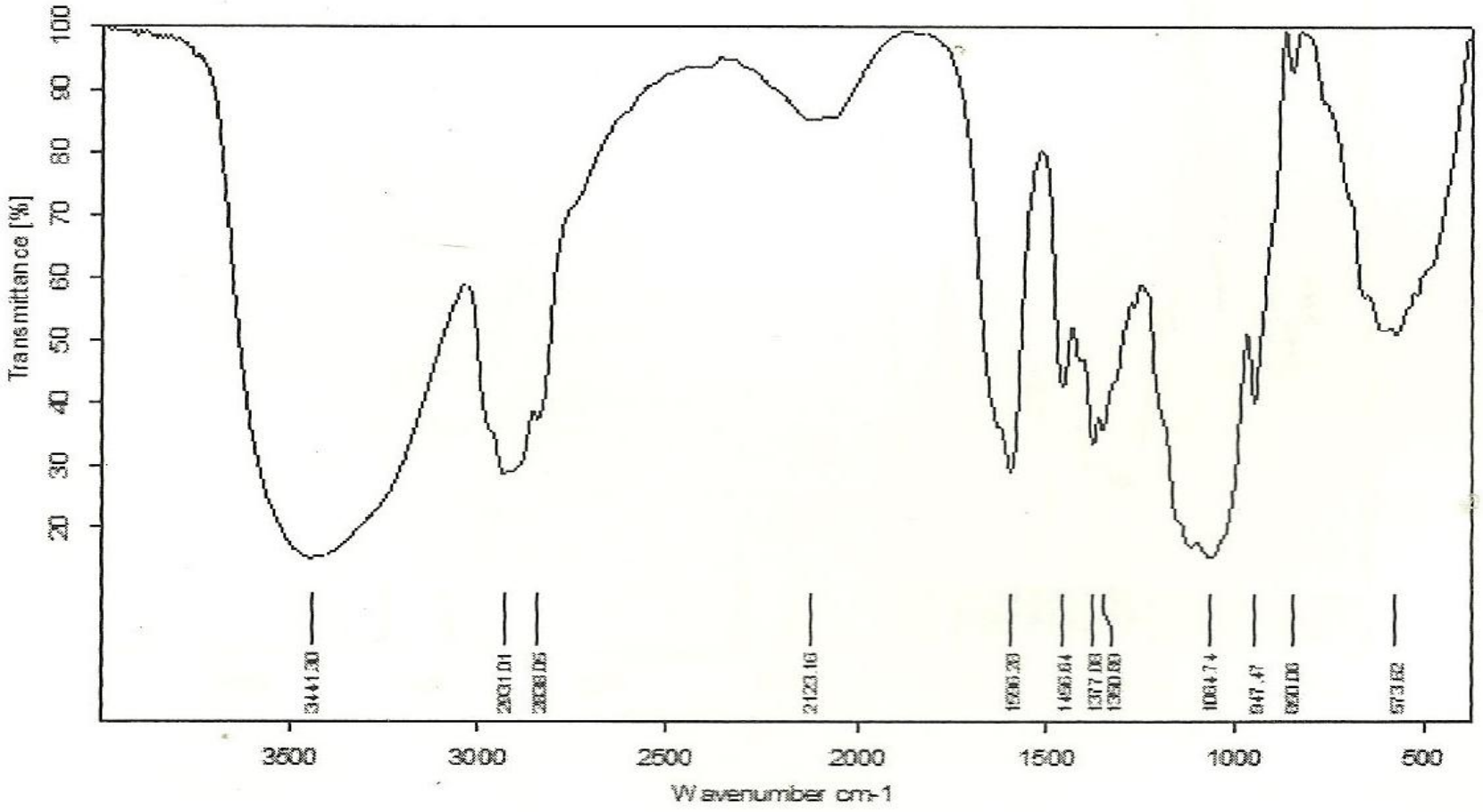


Figure No.9.3: IR Spectrum of HPMC K 100

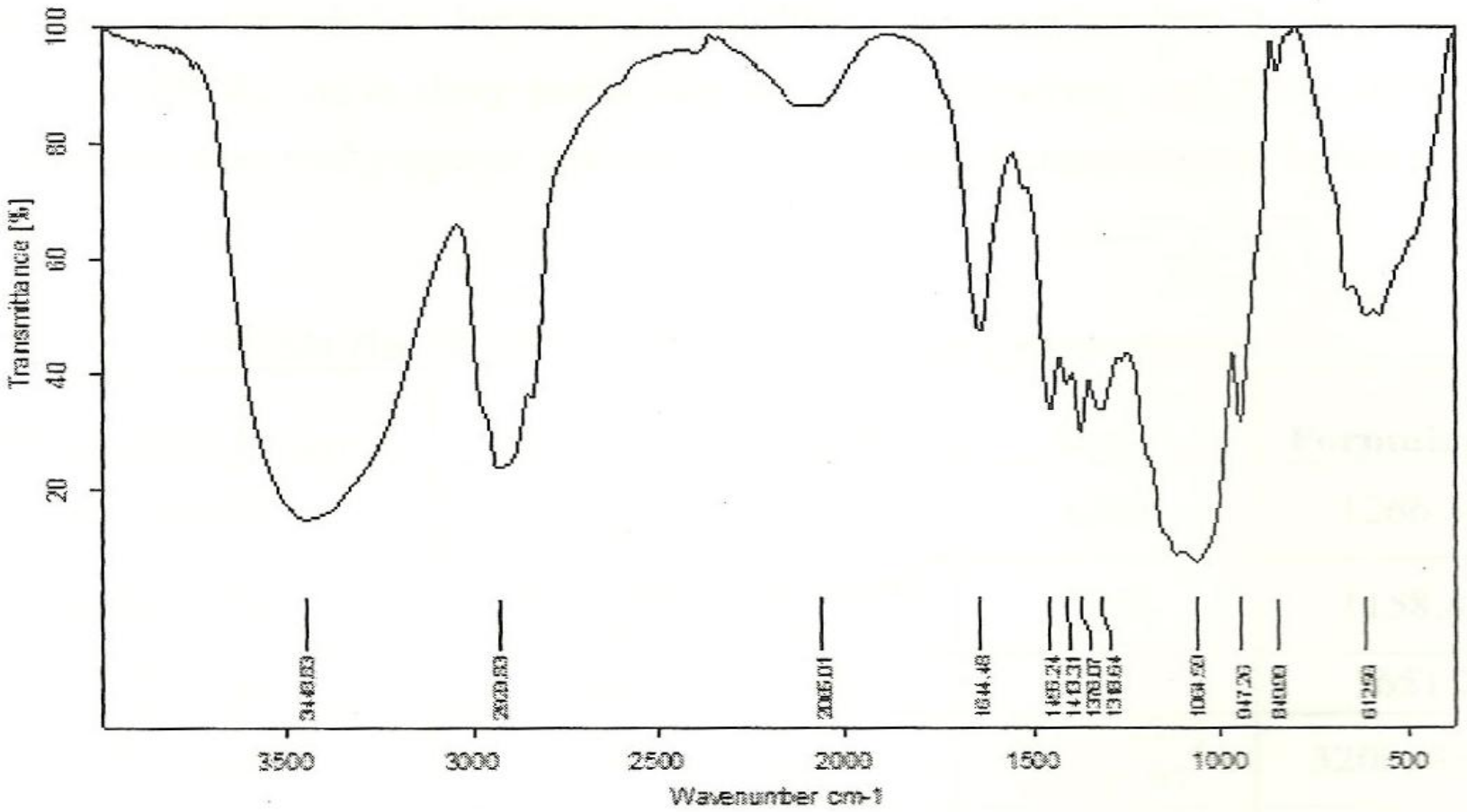


Figure No.9.4: IR Spectrum of HPMC K 15

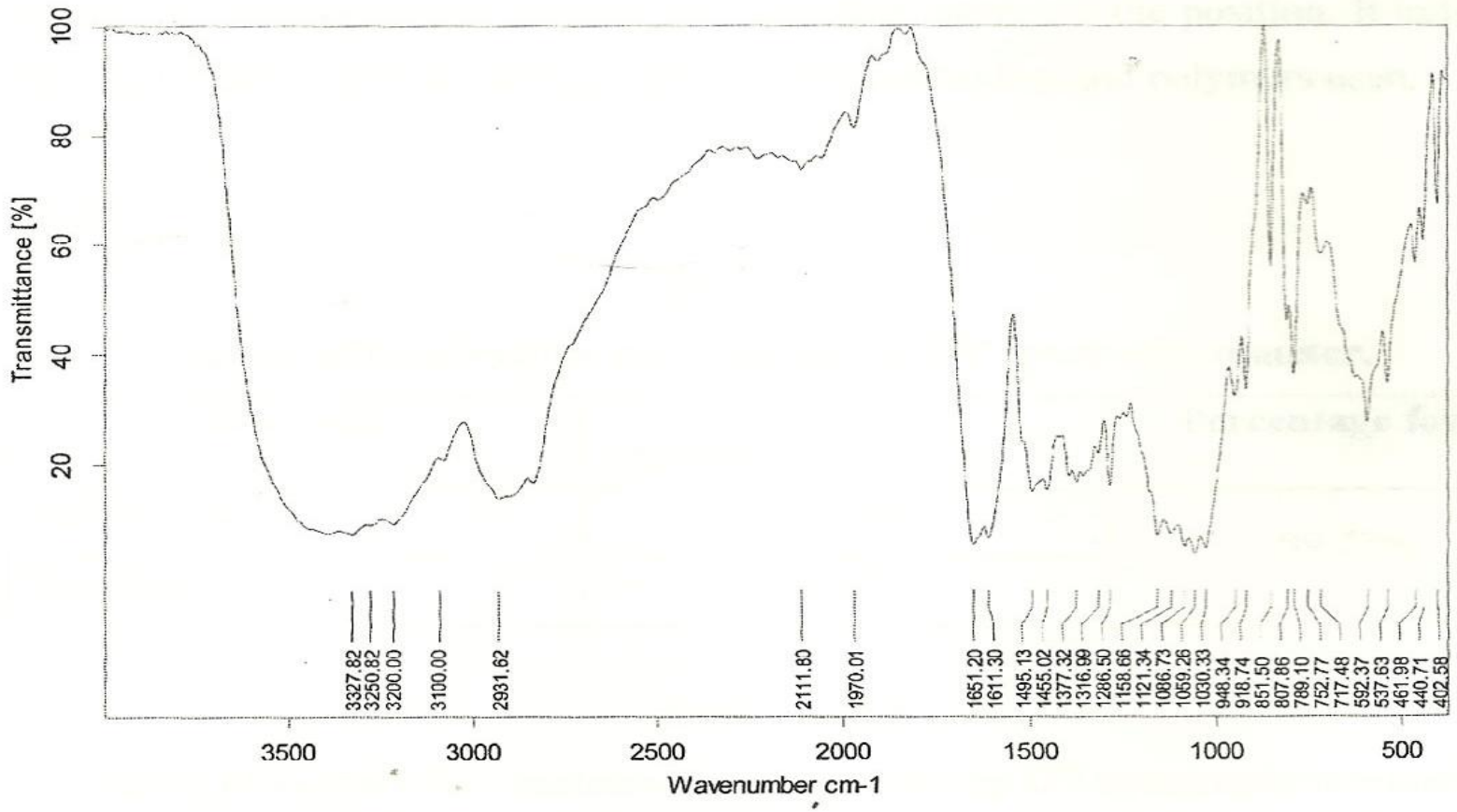


Figure No.9.5: IR Spectrum of physical mixture (Lamivudine+ HPMC K 100+ HPMC K 15)

The FTIR spectra of the pure drug, excipient and physical mixture of drug and excipient were recorded in between 400-2000 wave number (cm^{-1}), no peak are observed with the main drug peak. The following spectrum and table shows IR spectrum for drug and polymer and the wave number of characteristics bands are same.

Table no.9.2: FT-IR Peak of various components:

Wave Number in cm-1	Characteristic bands	Drug	Physical Mixture
1000-1300	Asymetric stretching of Ether system	1284	1266.34
1000-1400	Asymetric stretching of Ether system	1161	1158.85
1735-1750	Carbonyl group	1650	1651.2
3200-3400	Hydroxyl group	3319,3271 and 3197	3200, 3100
3300-3500	Amino group	3319, 3271 and 3197	3321, 3250

In FT-IR study the characteristic peak due to pure Lamivudine has appeared in the spectra of formulation without any markable changes in the position. It indicated that there was no chemical interaction between Lamivudine and polymer used.

EVALUATION PARAMETERS:

Table No.9.3: Evaluation of Lamivudine Blend.

Formulation code	Angle of repose (n=3)	Bulk density g/ml (n=3)	Tapped density (g/ml) (n=3)	Compressibility index (%) (n=3)	Hausner's ratio (n=3)
LF1	28^0.87"±1.20	0.466±0.002	0.542±0.012	14.02±0.92	1.16±0.07
LF2	27^0.95"±1.120	0.462±0.004	0.523±0.022	11.66±0.96	1.13±0.06
LF3	28^0.87"±1.08	0.463±0.004	0.542±0.013	14.75±0.88	1.17±0.04
LF4	27^0.91"±1.20	0.442±0.005	0.521±0.017	15.16±1.0	1.17±0.08
LF5	27^0.89"±1.14	0.431±0.003	0.501±0.015	13.9±1.12	1.16±0.05
LF6	28^0.79"±1.08	0.452±0.002	0.515±0.016	12.23±1.2	1.13±0.05
LF7	27^0.78"±1.12	0.449±0.003	0.522±0.013	13.98±1.04	1.16±0.04
LF8	28^0.89"±1.08	0.445±0.002	0.521±0.014	14.58±0.88	1.17±0.06
LF9	27^0.91"±1.10	0.462±0.004	0.524±0.013	11.83±0.98	1.13±0.07

±SD of means of three Determintion.

For the granules of all the formulated batches, the angle of repose was found to be in the range of 27° to 30°, thus indicating that the flow properties were good. Hausner's ratio was less than 1.18 for all the batches indicating good flow properties.

B] FOR ZIDOVUDINE:

Calibrations curve of Zidovudine in Phosphate buffer pH 6.8:

The calibration curve of the Zidovudine was prepared in the Phosphate buffer pH 6.8. Following **Table No 9.4** shows the absorbance at λ_{max} 267 nm and **Figure No. 9.6** Shows the calibration curve with regression coefficient 0.9999 , slope 0.0393 and the Y intercept +0.0003.

Table No.9.4: Data for Standard Curve of Zidovudine.

Sr.no	Concentration(µg/ml)	Absorbance at 267nm
1	5	0.199
2	10	0.388
3	15	0.595
4	20	0.783
5	25	0.983

Calibration Curve of Zidovudine.

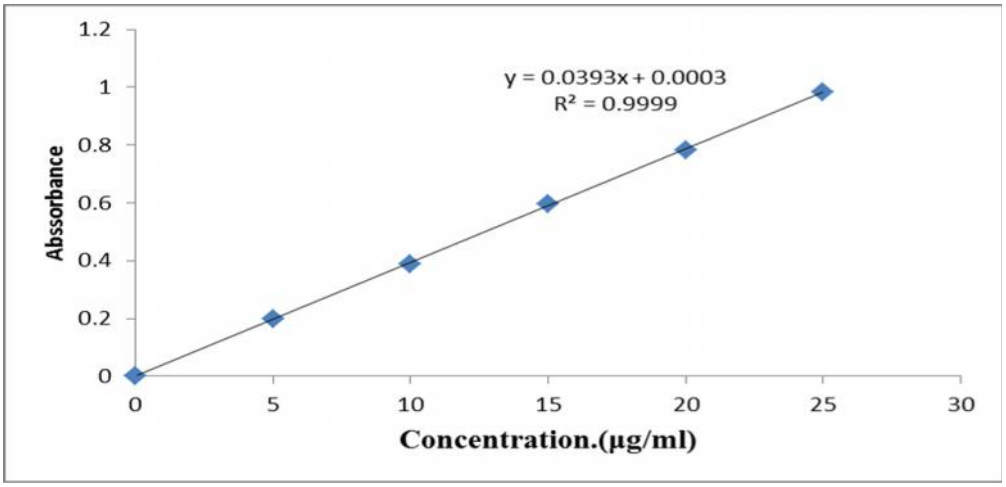


Figure No.9.6: Calibration curve Graph of Zidovudine in PH 6.8 Phoshphate Buffer.

Physical Evaluation of Zidovudine:-

Appearance, colour:

The powder sample was found to be white and crystalline.

Solubility:

The Zidovudine powder was found to be sparingly soluble in water, and freely soluble in methanol.

Identification Test:

The ratio of the absorbance measured at maxima 226nm to 267nm was found. That conform the sample taken was Zidovudine.

Assay:

The obtained sample of Zidovudine was found to be 98.94% pure by reported assay method.

Melting point:

The melting point was found to be 106-112^{0}C.

UV-Visible spectroscopy:

The Zidovudine was dissolved in methanol shows absorbance maximum at 267nm.

COMPATIBILITY STUDIES OF DRUG AND POLYMERS:

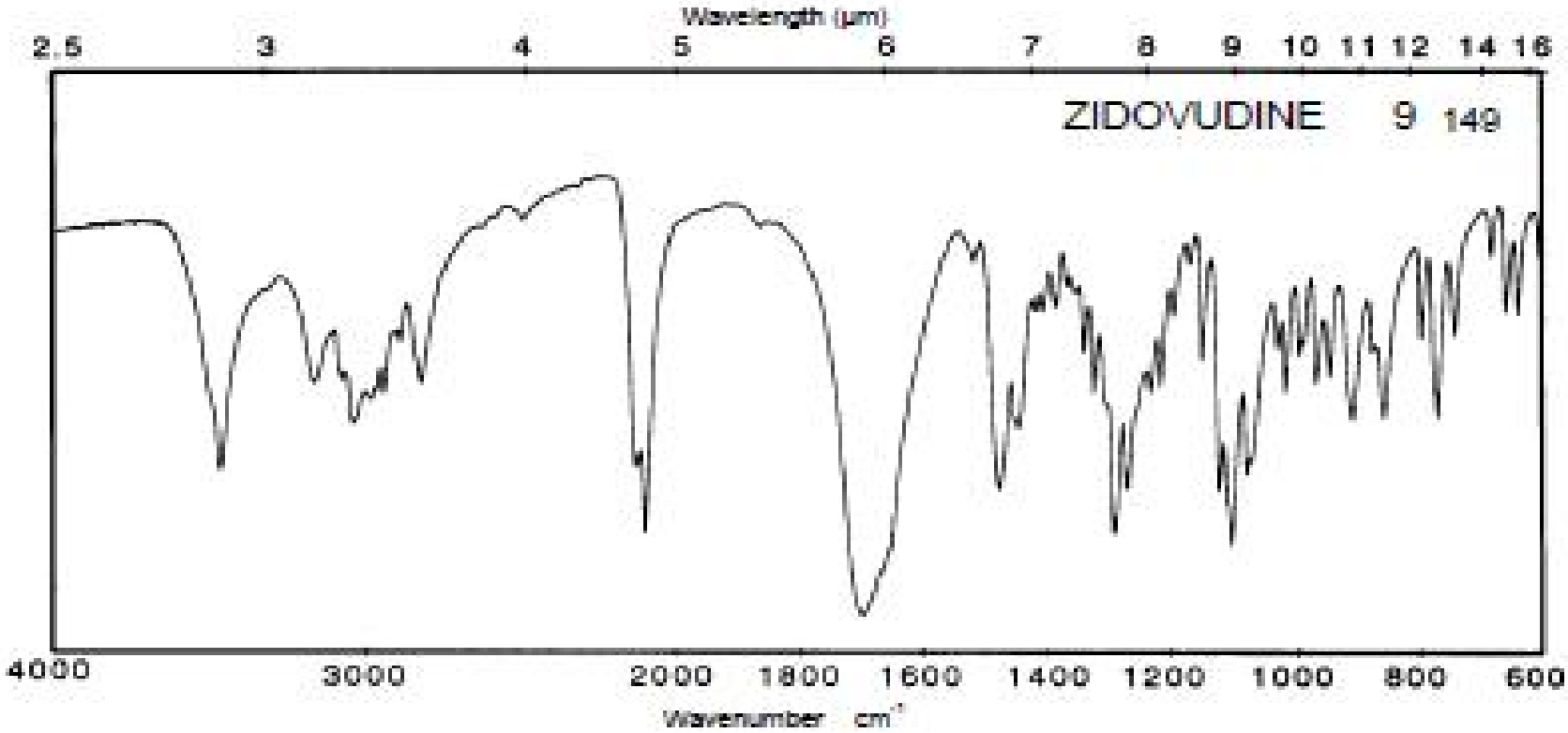


Figure No.9.7: IR Spectrum of pure Zidovudine Drug.

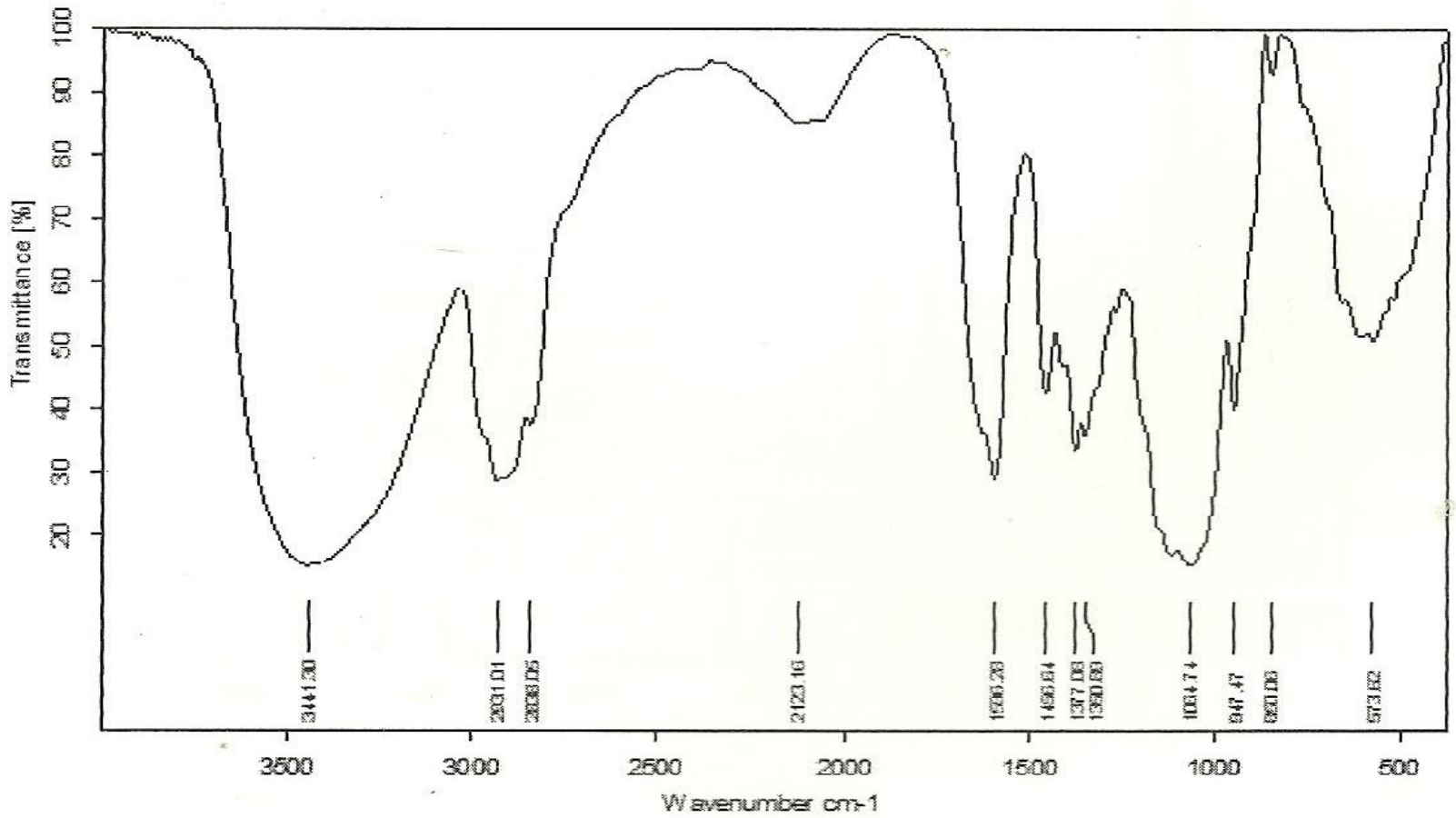


Figure No.9.8: IR Spectrum of HPMC K 100

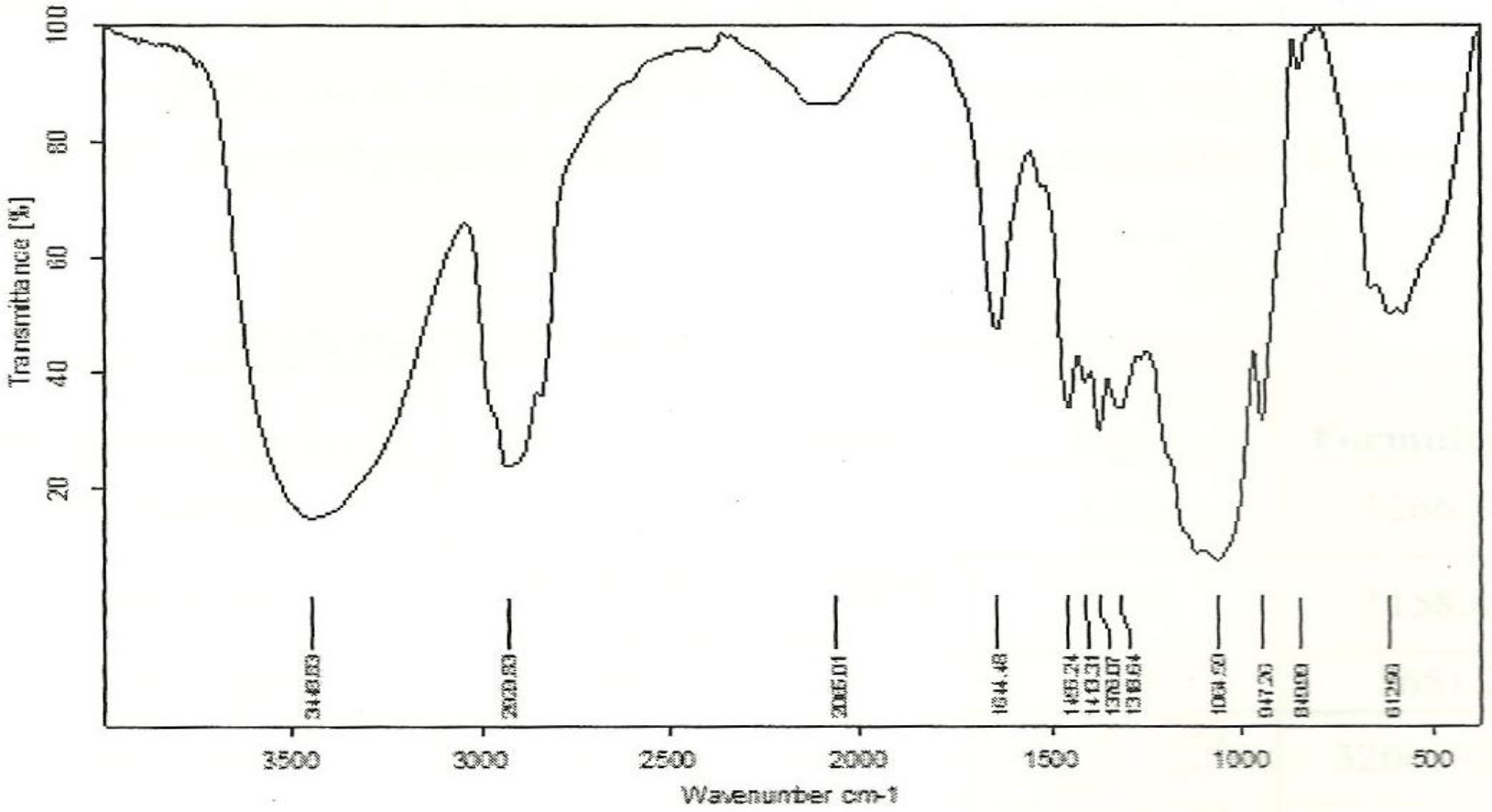


Figure No.9.9: IR Spectrum of HPMC K 15

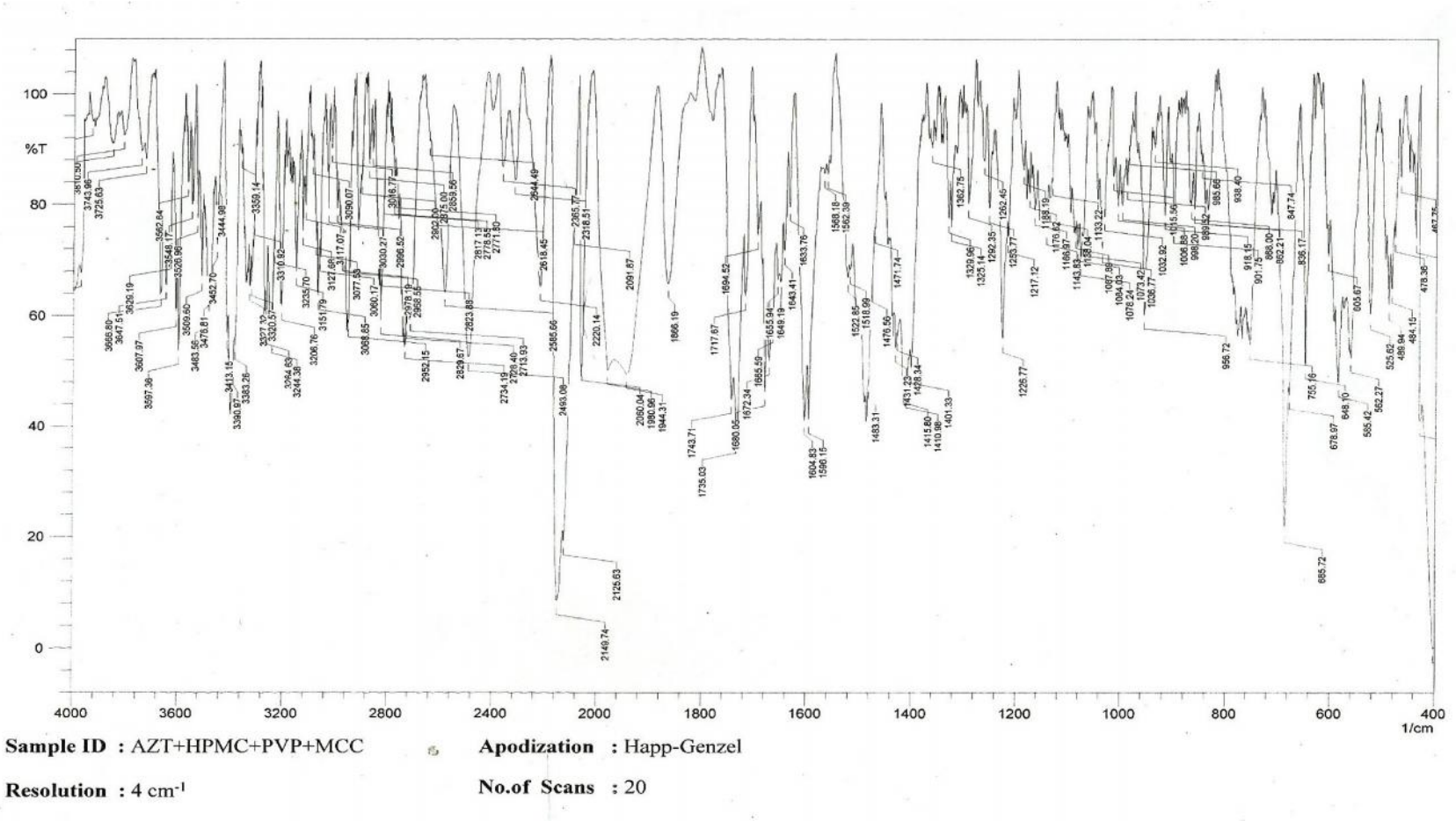


Figure No.9.10: FT-IR Spectrum of Physical Mixture (Zidovudine+ HPMC K100+HPMC K15+PVP+MCC)

Table No.9.5: FT-IR Peak of various components:

Wave Number in cm-1	Characteristic bands	Drug	Physical Mixture
1000-1300	Asymetric stretching of Ether system	1284	1266.34
1000-1400	Asymetric stretching of Ether system	1161	1158.85
1735-1750	Carbonyl group	1695	1676.7
3200-3400	Hydroxyl group	3319,3271 and 3197	3200, 3100
3300-3500	Amino group	3319, 3271 and 3197	3321, 3250

In FT-IR study the characteristic peak due to pure Zidovudine has appeared in the spectra of formulation without any markable changes in the position. It indicated that there was no chemical interaction between Zidovudine and polymer used.

EVALUATION PARAMETERS:

Table No.9.6: Evaluation of Zidovudine Blend.

Formulation code	Angle of repose (n=3)	Bulk density (g/ml) (n=3)	Tapped density (g/ml) (n=3)	Compressibility index (%) (n=3)	Hausner's ratio (n=3)
ZF1	29^0.81"±0.79	0.425±0.002	0.487±0.012	12.73±0.96	1.14±0.05
ZF2	28^0.85"±0.91	0.408±0.004	0.465±0.015	12.25±0.90	1.13±0.03
ZF3	27^0.83"±0.92	0.377±0.006	0.434±0.010	13.13±0.92	1.15±0.04
ZF4	28^0.87"±0.87	0.363±0.003	0.408±0.013	11.02±0.94	1.12±0.07
ZF5	27^0.91"±0.84	0.370±0.003	0.418±0.011	11.48±0.91	1.12±0.04
ZF6	29^0.93"±0.78	0.368±0.002	0.422±0.014	12.79±1.04	1.14±0.01
ZF7	28^0.85"±0.87	0.362±0.006	0.414±0.013	12.56±0.98	1.14±0.03
ZF8	29^0.86"±0.84	0.364±0.005	0.416±0.012	12.5±0.94	1.14±0.04
ZF9	28^0.81"±0.88	0.360±0.007	0.412±0.017	12.62±0.96	1.14±0.02

±SD of mean of three Determination

For the granules of all the formulated batches, the angle of repose was found to be in the range of 25^0 to 30^0, thus indicating that the flow properties were good. Hausner's ratio was less than 1.25 for all the batches indicating good flow properties.

POST-FORMULATION STUDY:-

The Tablet from each formulation were evaluated for Uniformity in Drug content, Average weight, Thickness, Hardness and result are reported in **Table No 9.7** .

The tablets showed good weight uniformity as indicated by the low value of Relative Standard Deviation (RSD< 1%), The Tablet thickness were found to range from 3.90 ± 0.01 mm to 4.00 ± 0.01 mm, The Drug content uniformity of the Tablet was found to comply with the official specification, as the assay value found to in range between 98.65 ± 0.62 to 101.28 ±1.37 of the theoretical value. The tablet Hardness varied from 6.0 ±0.2 to 6.5 ± 0.4. The tablets passed the friability test, as all the batches were within the pharmacopoeial limit (F< 1%).

Table No.9.7: Various properties of Tablet including average weight, Thickness, Hardness, Friability & % Drug Content.

Batch code	Average weight (mg) [n=20]	Thickness (mm) [n=3]	Hardness Kg/cm^2 [n=3]	% Friability	Percentage Drug Content [n=3]	
					Lamivudine	Zidovudine
LF1+ZF1	587±4.89	3.90± 0.01	6.5 ± 0.3	0.18	99.54 ±0.78	98.61 ± 0.34
LF2+ZF2	598.25±4.75	3.90± 0.02	6.0 ± 0.6	0.23	100.85 ± 1.03	99.1 ± 0.92
LF3+ZF3	609.5±3.76	3.94± 0.02	6.5 ± 0.2	0.23	98.85 ± 0.36	99.22 ± 0.78
LF4+ZF4	632±4.74	3.94± 0.01	6.0 ± 0.2	0.22	101.07 ± 1.12	100.05 ± 0.97
LF5+ZF5	654.5±3.85	3.96±0.02	6.5 ± 0.4	0.19	99.14 ± 1.07	98.93 ± 0.54
LF6+ZF6	677±2.85	4.00± 0.01	6.0 ± 0.3	0.29	101.19 ± 0.93	99.87 ± 0.76
LF7+ZF7	609.5±4.07	3.94± 0.01	6.0 ± 0.2	0.28	98.65± 0.62	99.45 ± 0.45
LF8+ZF8	626.62±4.36	3.94± 0.02	6.5 ± 0.2	0.34	100.25 ±1.51	101.12 ± 0.1.04
LF9+ZF9	643.25±3.92	3.98± 0.01	6.0 ± 0.4	0.27	101.28 ±1.37	100.12 ± 0.98

***In Vitro* Evaluation of Controlled release Bilayer Matrix Tablet:**

A] *In vitro* Dissolution Studies of Lamivudine Layer from formulation LF1-LF9 in Phosphate buffer pH 6.8.

Table No.9.8:

Time (Hrs.)	Cumulative Percentage Drug release								
	LF1	**LF2**	**LF3**	**LF4**	**LF5**	**LF6**	**LF7**	**LF8**	**LF9**
0	**0**	**0**	**0**	**0**	0	**0**	**0**	**0**	**0**
1	25.02	24.44	23.03	23.22	20.32	18.55	22.65	21.18	20.25
2	39.12	37.32	36.13	34.19	32.31	30.43	35.14	33.45	32.61
4	54.21	50.11	49.21	49.26	48.76	44.05	49.31	48.27	45.36
6	67.03	62.15	59.34	64.47	62.55	60.16	64.45	62.65	61.72
8	83.43	81.33	80.65	77.13	74.45	71.48	77.67	73.71	71.88
10	97.04	94.23	92.12	93.34	85.56	82.71	88.12	84.29	80.36
12	-	-	-	-	97.79	93.09	96.35	95.09	91.73

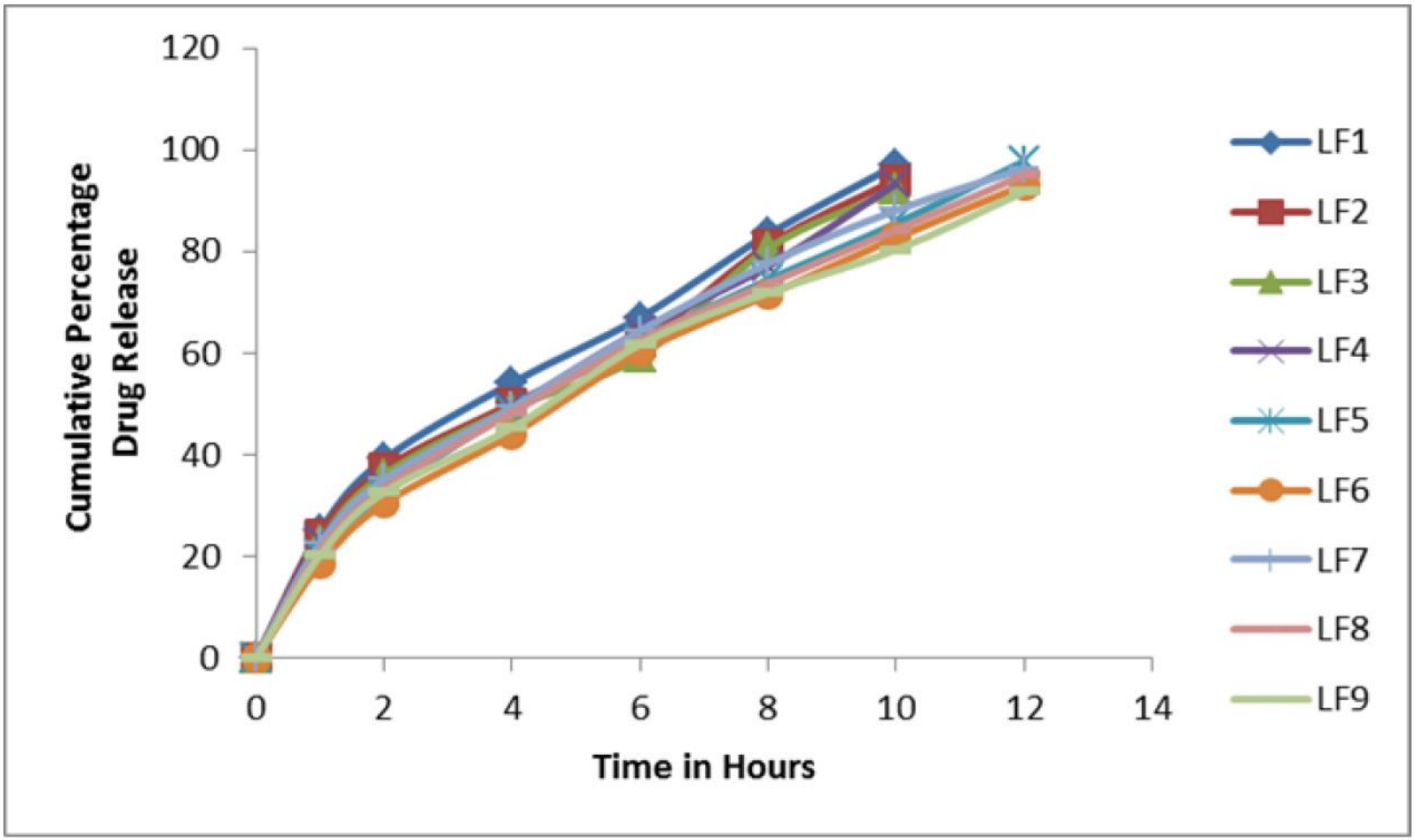


Figure No 9.11: Cumulative Percentage Drug release of formulation LF1-LF9.

B] *In vitro* Dissolution Studies of Zidovudine Layer from formulationZF1-ZF9 in Phosphate buffer pH 6.8:

Table No.9.9:

Time (Hrs.)	Cumulative Percentage Drug release								
	ZF1	**ZF2**	**ZF3**	**ZF4**	**ZF5**	**ZF6**	**ZF7**	**ZF8**	**ZF9**
0	0	0	0	0	0	0	0	0	0
1	27.44	26.12	25.78	26.42	24.29	20.67	25.7	24.30	22.17
2	40.16	39.47	36.39	39.11	37.43	30.82	38.38	36.42	33.72
4	55.29	51.37	52.76	55.19	52.13	45.39	54.53	52.34	51.14
6	68.41	66.03	62.47	67.34	65.82	59.51	67.71	64.19	61.32
8	82.62	80.18	79.48	80.72	76.55	71.63	81.37	75.24	72.63
10	97.07	95.37	93.17	94.21	88.38	84.26	91.32	86.62	84.27
12	_	_	_	_	98.68	92.09	96.05	94.17	93.63

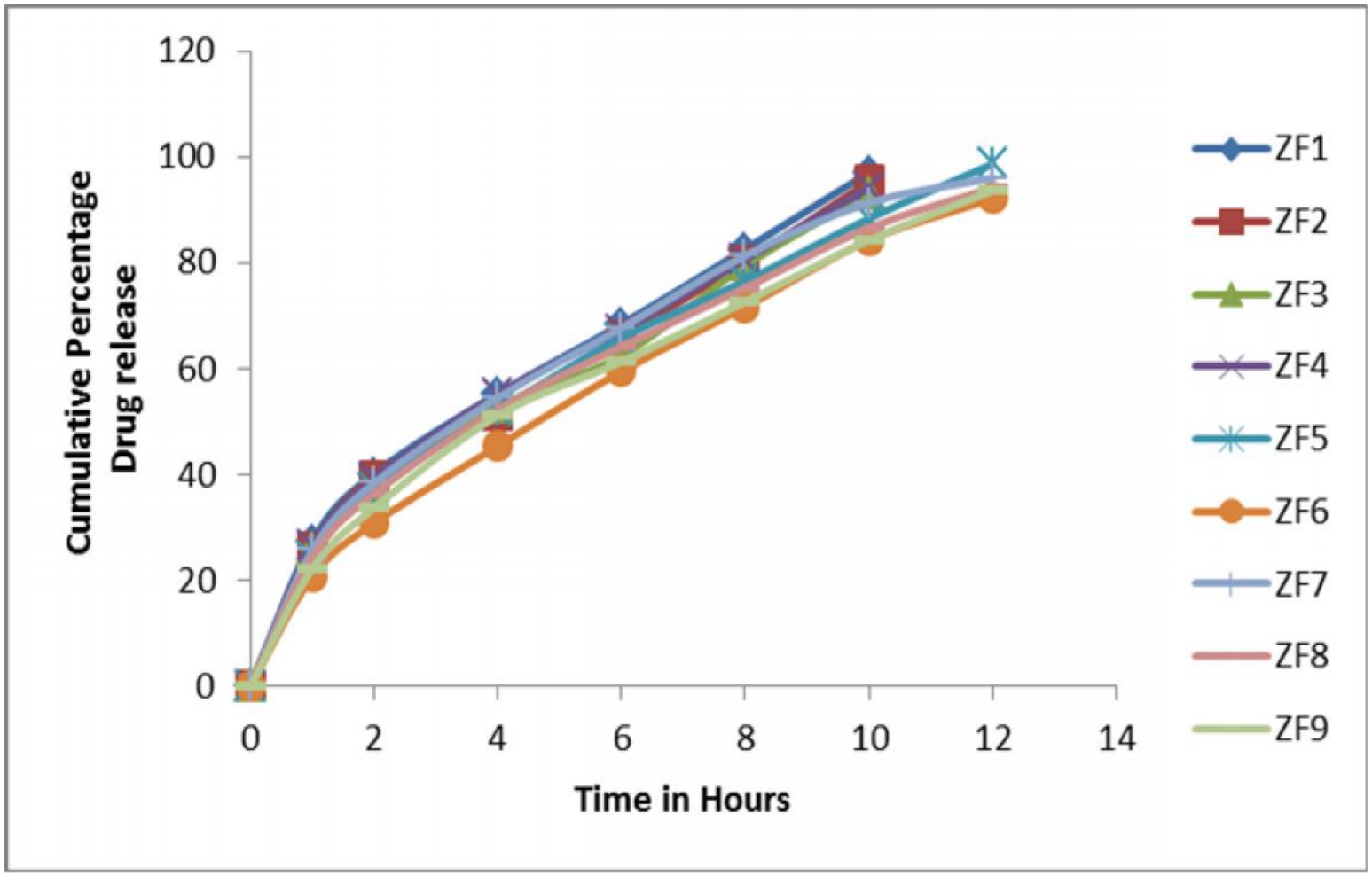


Fig No 9.12: Cumulative Percentage Drug release of formulation ZF1-ZF9.

HPLC STUDY FOR PURE LAMIVUDINE AND ZIDOVUDINE DRUG:

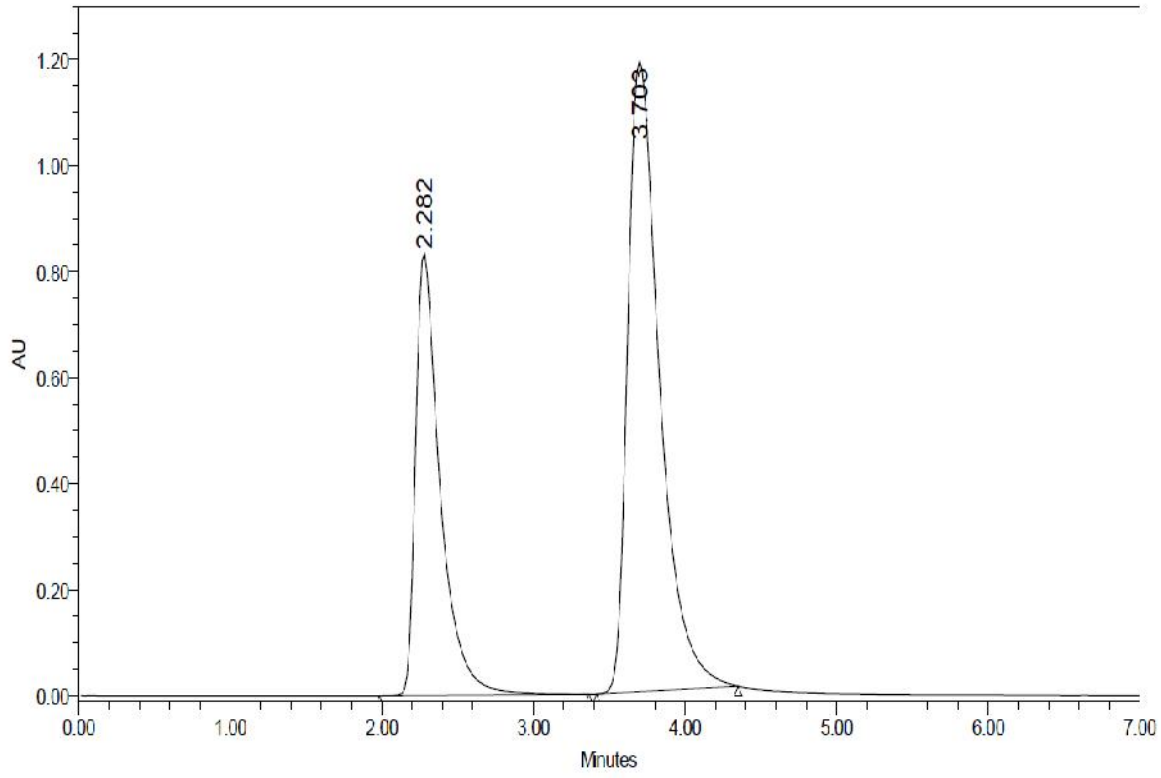


Figure No.9.13: Chromatogram of Pure Lamivudine and Zidovudine Drug.

Table No.9.10: Data obtained from pure Lamivudine and Zidovudine Drug.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	lam-1	2.28	9366159	834328	1088.1		1.8
2	lam-2	3.70	17334310	1191873	1611.8	4.2	1.6

HPLC STUDY FOR BEST FORMULATION (LF5+ZF5):

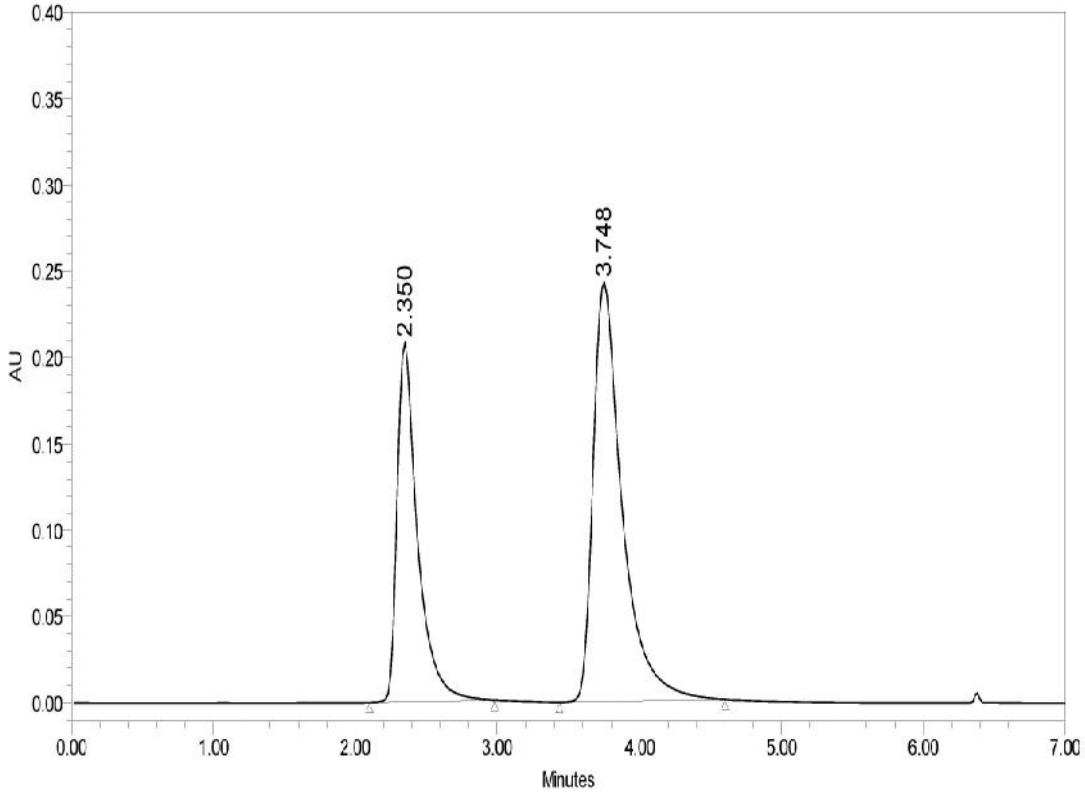


Figure No.9.14: Peak obtained after 1hr dissolution.

Table No.9.11:Data obtained from HPLC after 1hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	Lamivudine	2.35	2055534	207601	1456.9		1.7
2	Zidovudine	3.75	4624665	242638	1852.7	4.5	1.7

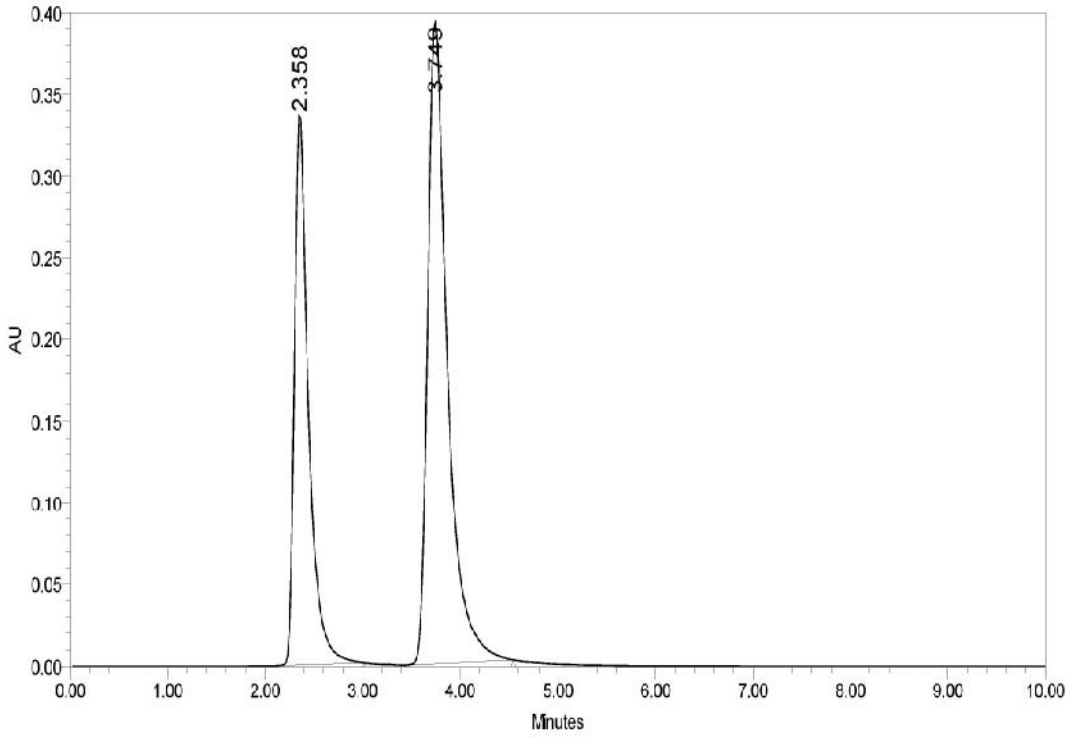


Figure No.9.15: Peak obtained after 2hr dissolution.

Table No.9.12: Data obtained from HPLC after 2hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.36	3355531	339007		1.7	1433
2	Zidovudine	3.75	7003257	393464	4.4	1.7	1872

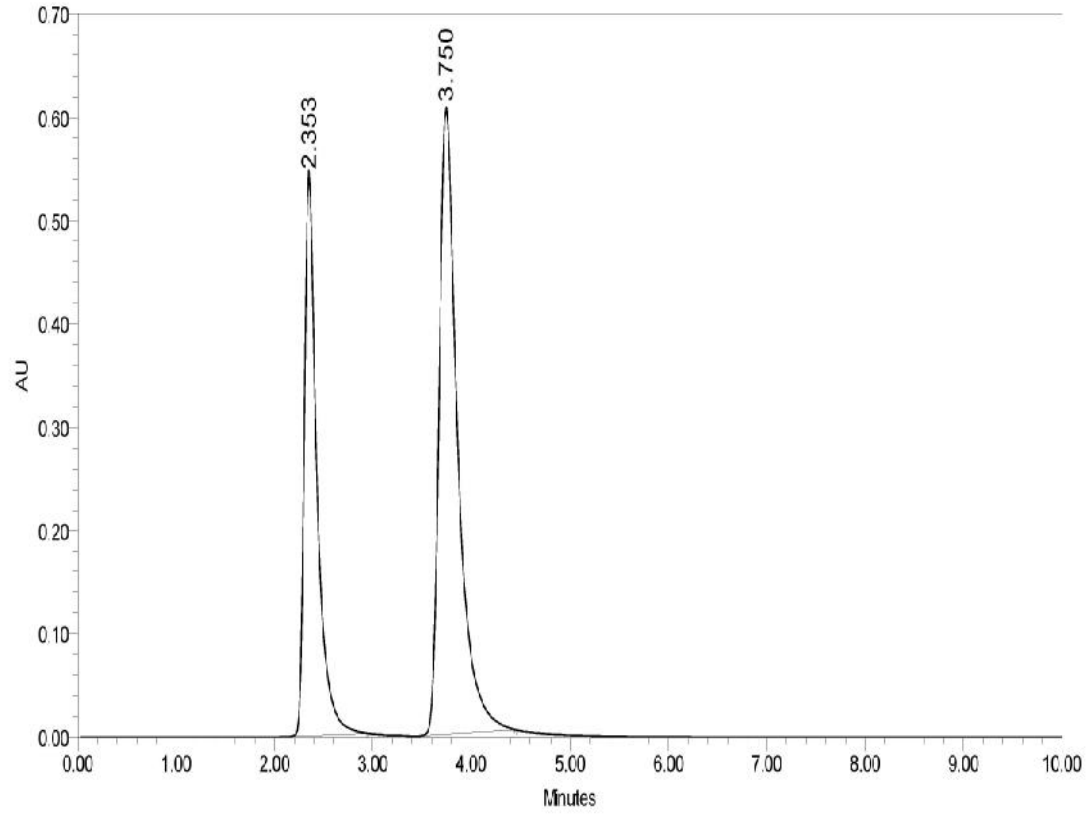


Figure No.9.16: Peak obtained after 4hr dissolution.

Table No.9.13: Data obtained from HPLC after 4hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Lamivudine	2.35	4957743	546783		1.7	1871
2	Zidovudine	3.75	9942861	606807	4.9	1.7	2102

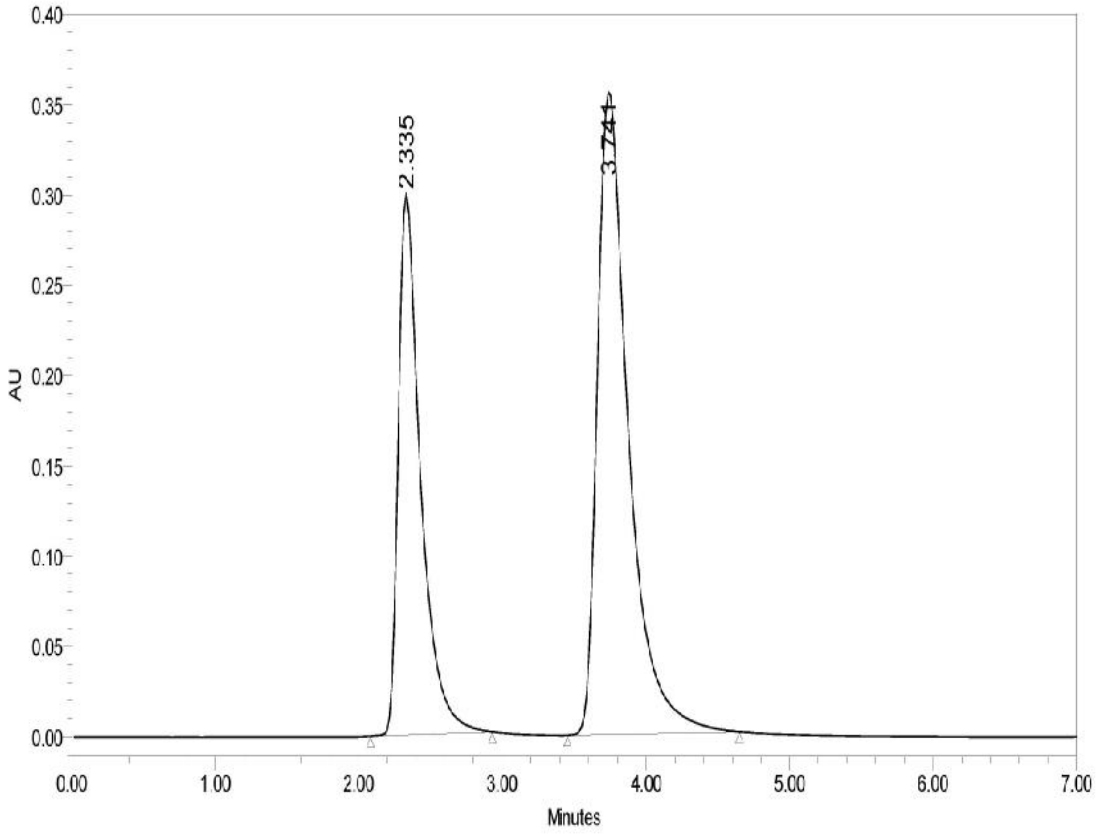


Figure No.9.17: Peak obtained after 6hr dissolution.

Table No.9.14: Data obtained from HPLC after 6hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	Lamivudine	2.34	6335569	299872	1155.7		1.8
2	Zidovudine	3.74	12394257	356952	1585.1	4.1	1.7

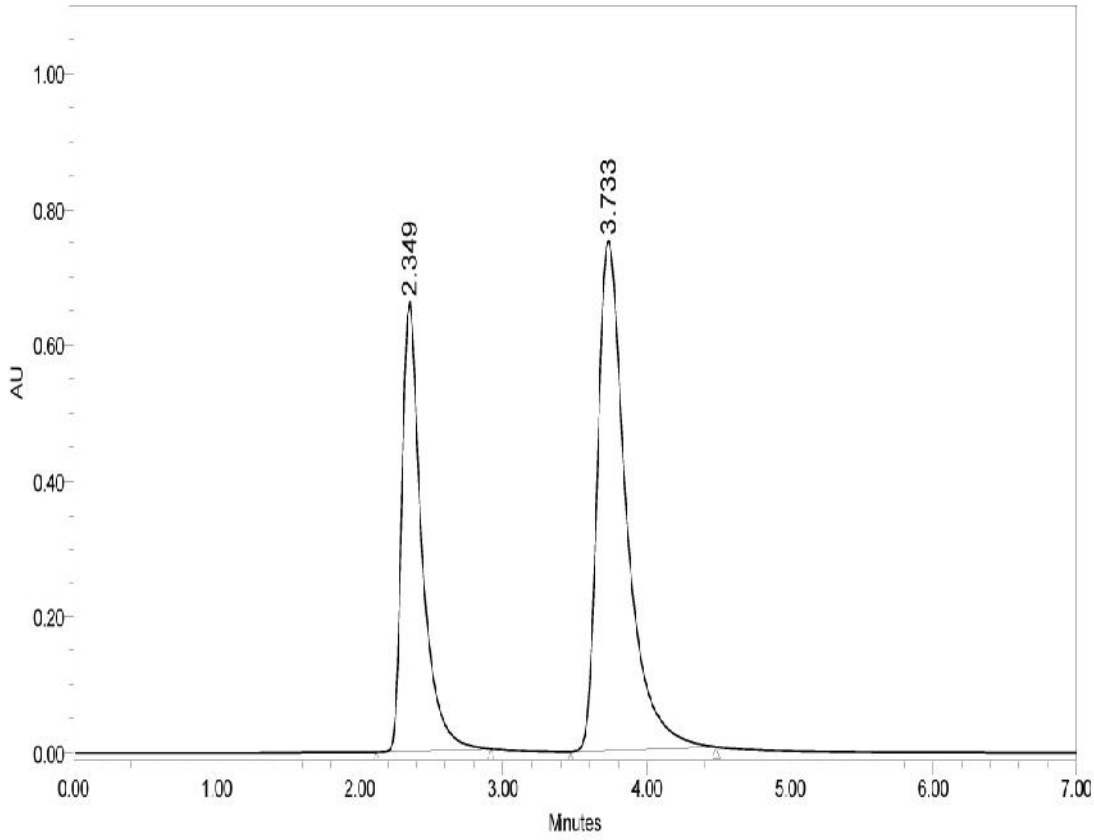


Figure No.9.18: Peak obtained after 8hr dissolution.

Table No.9.15: Data obtained from HPLC after 8hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	Lamivudine	2.35	7521065	660214	1555.0		1.7
2	Zidovudine	3.73	14296531	750992	1845.8	4.5	1.7

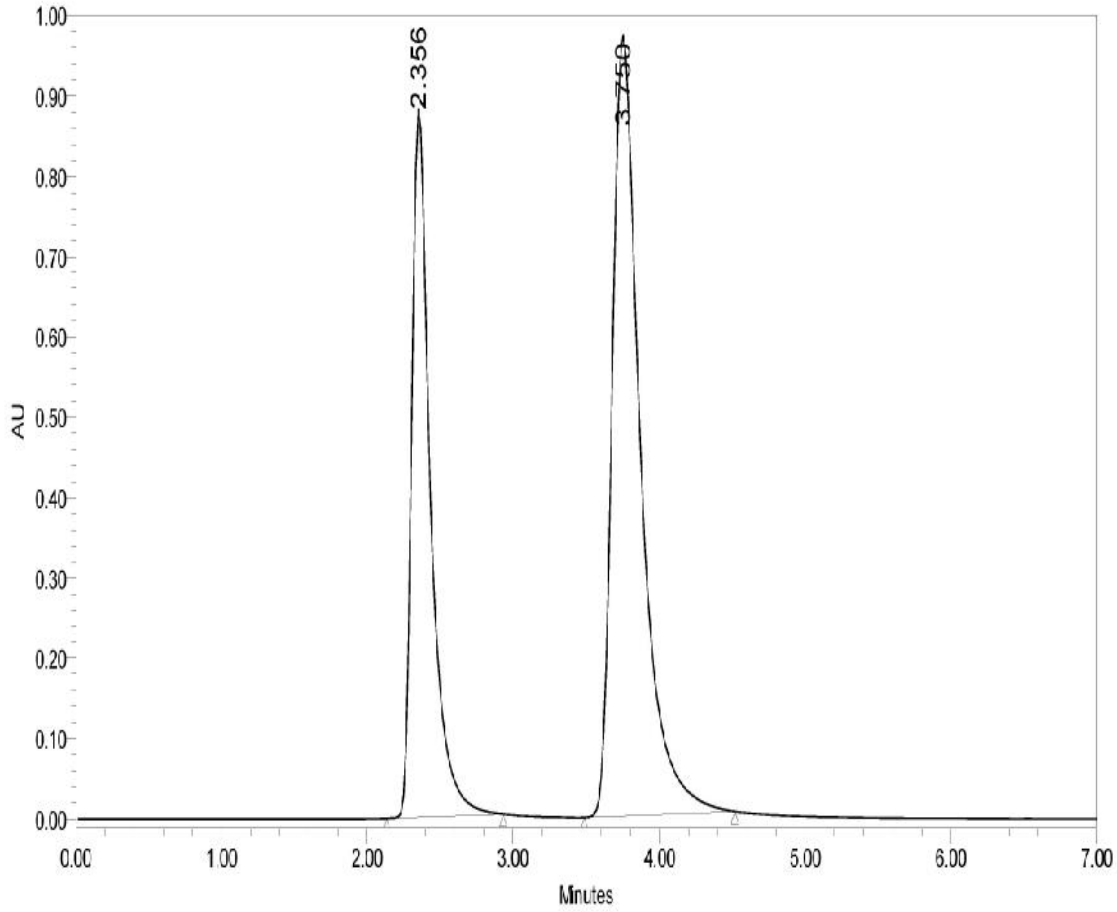


Figure No.9.19: Peak obtained after 10hr dissolution.

Table No.9.16: Data obtained from HPLC after 10hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	Lamivudine	2.36	8553959	884052	1857.6		1.7
2	Zidovudine	3.75	16379356	971476	2015.0	4.8	1.7

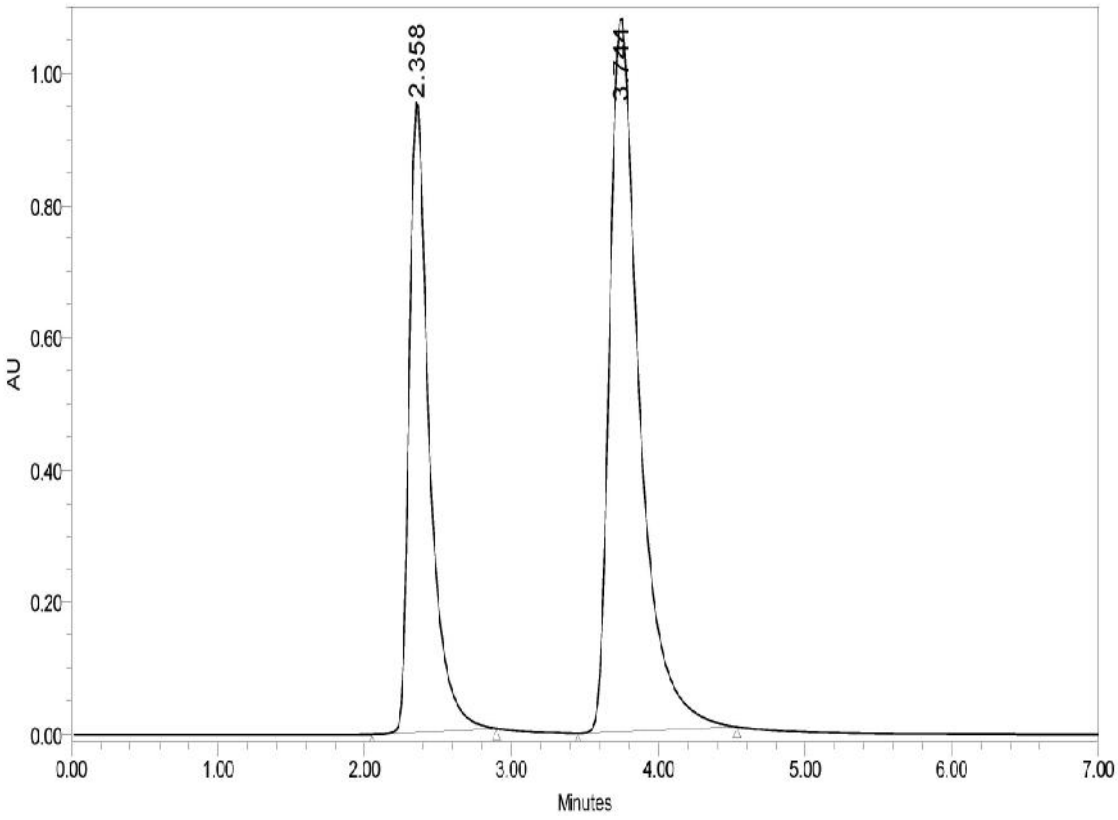


Figure No.9.20: Peak obtained after 12hr dissolution.

Table No.9.17: Data obtained from HPLC after 12hr dissolution.

	Name	Retention Time (min)	Area (µV*sec)	Height (µV)	USP Plate Count	USP Resolution	USP Tailing
1	Lamivudine	2.36	9724320	961431	1585.3		1.7
2	Zidovudine	3.74	18220610	1079946	1838.2	4.5	1.7

KINETIC STUDY OF LAMIVUDINE AND ZIDOVUDINE BILAYER TABLET (LF5+ZF5):

A] FOR LAMIVUDINE LAYER FORMULATION (LF5):

Table No.9.18:

Time in Hrs.	√Time (Hrs.)	Log Time (Hrs.)	Cumulative Percentage Drug Release	Log Cumulative Percentage Drug Release	Percentage Drug Remained	Log Percentage Drug Remained
0	0.00	0.00	0	0	100	2.00
1	1.0	0.00	20.32	1.3080	79.68	1.9013
2	1.41	0.3010	32.31	1.5093	67.69	1.8305
4	2.00	0.6021	48.76	1.6880	51.24	1.7096
6	2.45	0.7782	62.55	1.7962	37.45	1.5734
8	2.83	0.9031	74.45	1.8718	25.55	1.4073
10	3.16	1.00	85.56	1.9322	14.44	1.1595
12	3.46	1.0792	97.79	1.9902	2.21	0.3443

Zero Order Plot

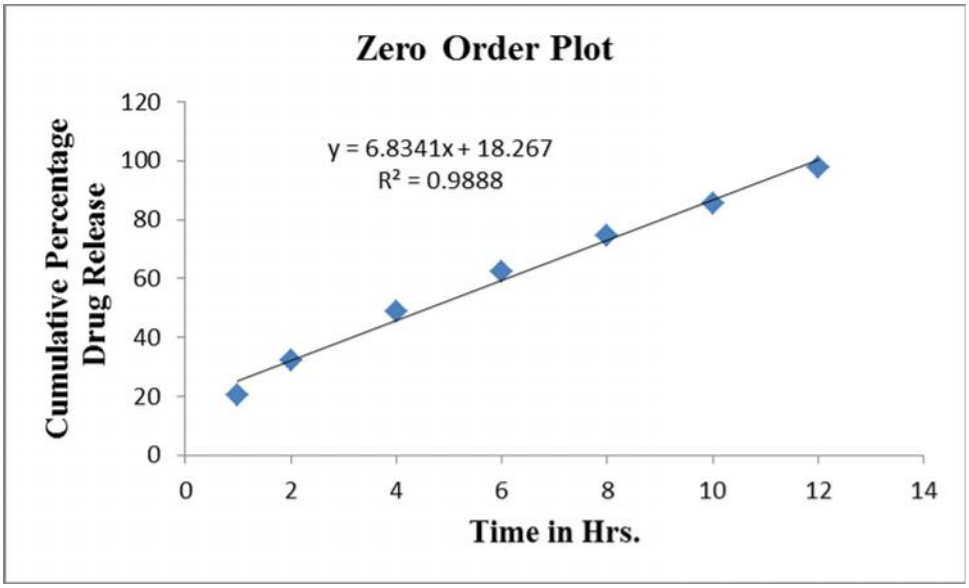


Figure No.9.21: Zero Order Plot

First Order Plot

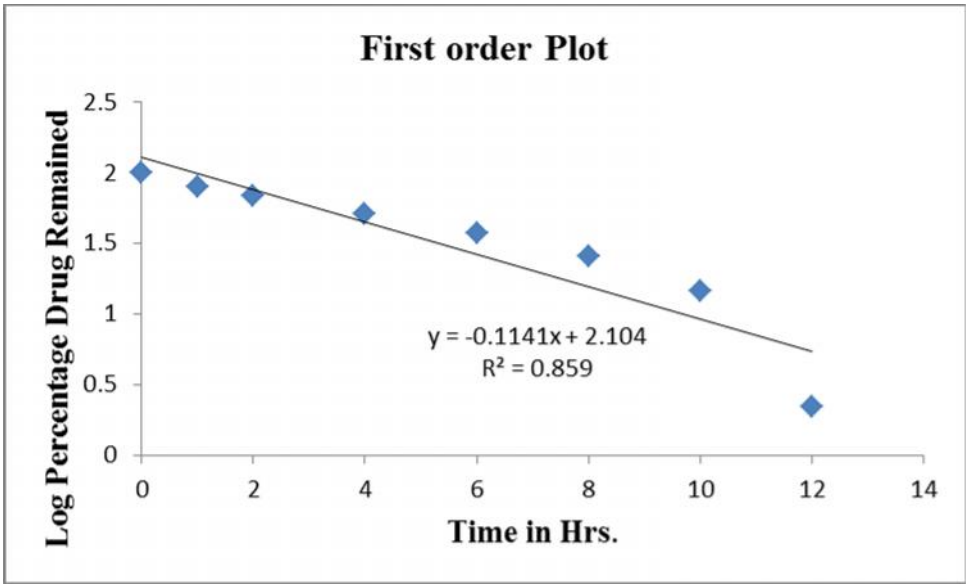


Figure No.9.22: First Order Plot

Higuchi Plot

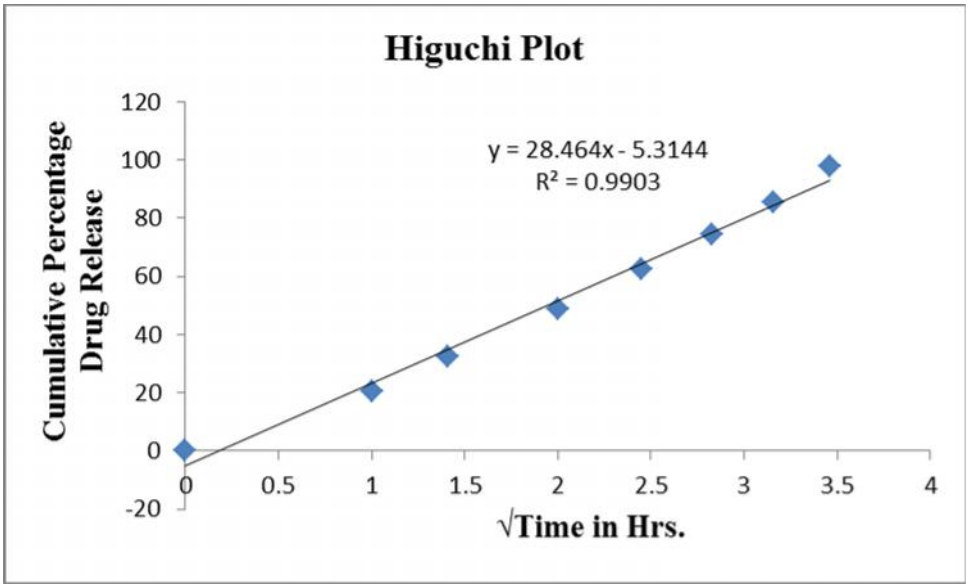


Figure No.9.23: Higuchi Plot

Korsmeyer peppas plot

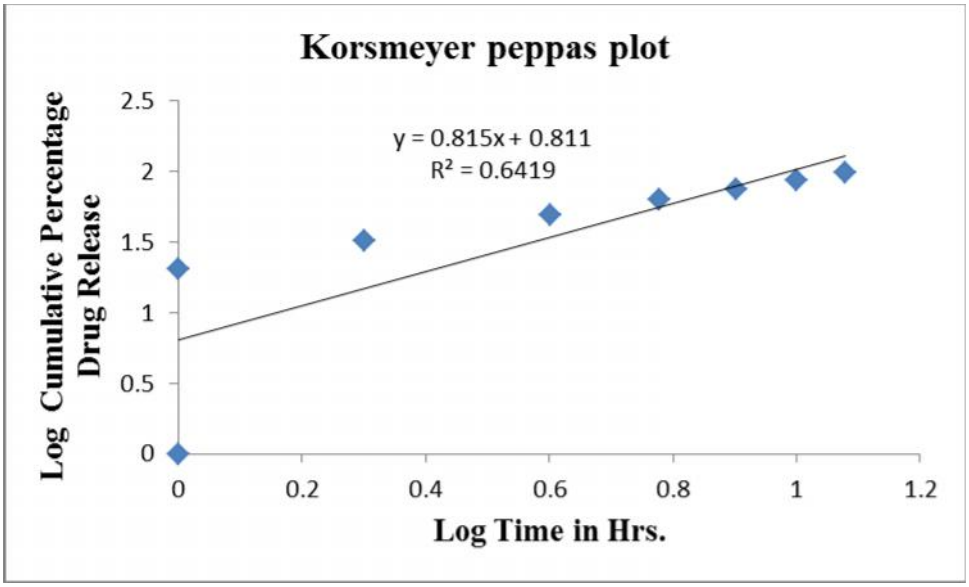


Figure No.9.24: Korsmeyer peppas plot.

Table No.9.19: Kinetic values obtained from Lamivudine Layer (LF5).

Formuation	Zero order plot R^2	First order plot R^2	Higuchi R^2	Korsmeyer-Peppas Plot R^2	N	Mechanism of Drug Release
LF5	0.9888	0.859	0.9903	0.6419	0.815	Zero order non fickian diffusion.

Mechanism of Drug Release

In order to understand the complex mechanism of drug releasevfrom the matrix system, the in vitro release rate were fitted to Korsmeyer-peppas model and interpretition of release component value (n) enlighten in understanding the release mechanism from the dosage form. The release exponent value (n) thus obtained was 0.815. The LF5 formulation exhibited anomalous (non-fickian) diffusion mechanism.

These formulations are also showed as highest R^2 values of zero order kinetics indicating the amount of drug from the matrix system were by both diffusion and erosion.

B] FOR ZIDOVUDINE LAYER FORMULATION (ZF5):

Table No. 9.20:

Time in Hrs.	√Time (Hrs.)	Log Time (Hrs.)	Cumulative Percentage Drug Release	Log Cumulative Percentage Drug Release	Percentage Drug Remained	Log Percentage Drug Remained
0	0.00	0.00	0	0	100	2.00
1	1.0	0.00	24.29	1.3854	75.71	1.8791
2	1.41	0.3010	37.43	1.5742	62.57	1.7963
4	2.00	0.6021	52.13	1.7170	47.87	1.6800
6	2.45	0.7782	65.82	1.8183	34.18	1.5337
8	2.83	0.9031	76.55	1.8839	23.45	1.3701
10	3.16	1.00	88.38	1.9463	11.62	1.0652
12	3.46	1.0792	98.68	1.9942	1.32	0.1205

Zero order plot

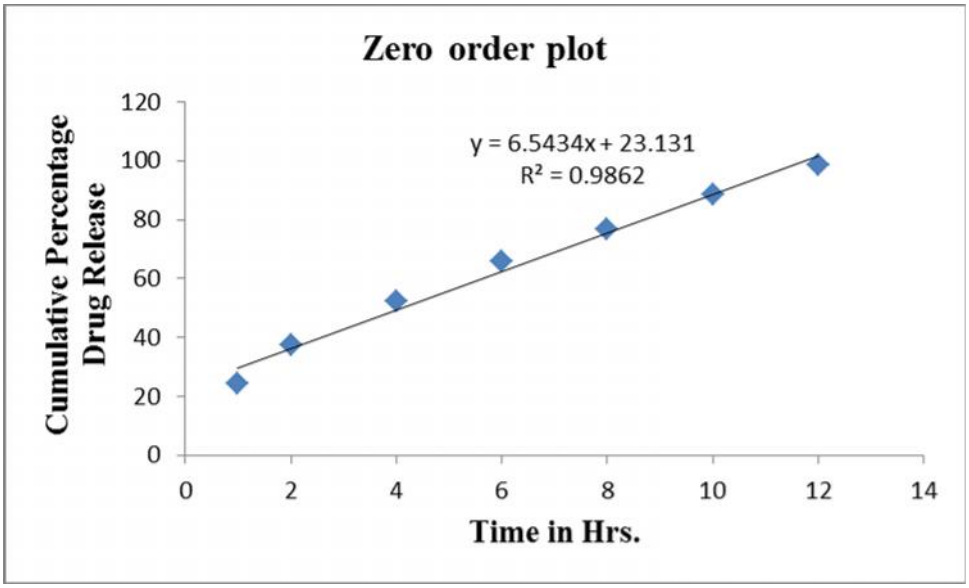


Figure No.9.25: Zero order plot

First order plot

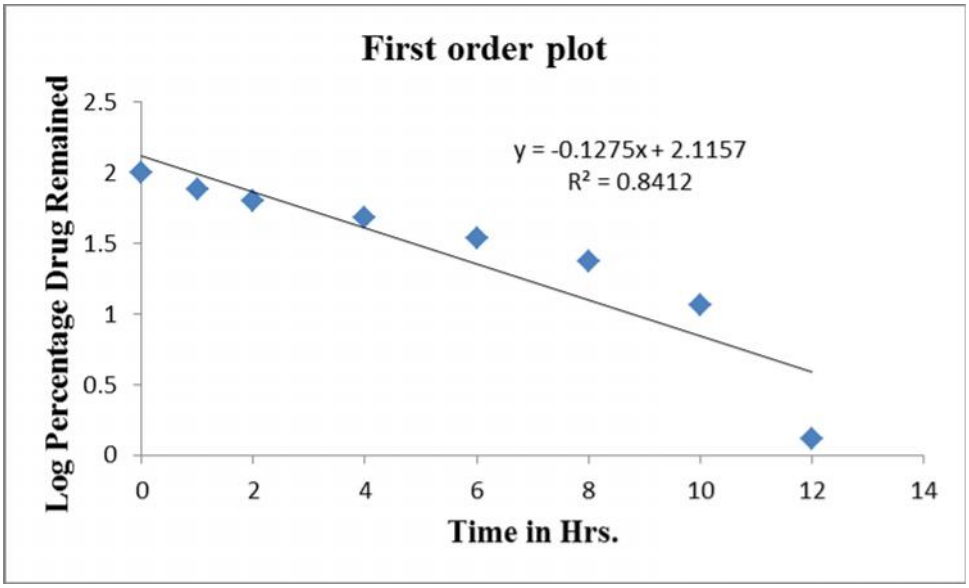


Figure No.9.26: First order plot

Higuchi Plot

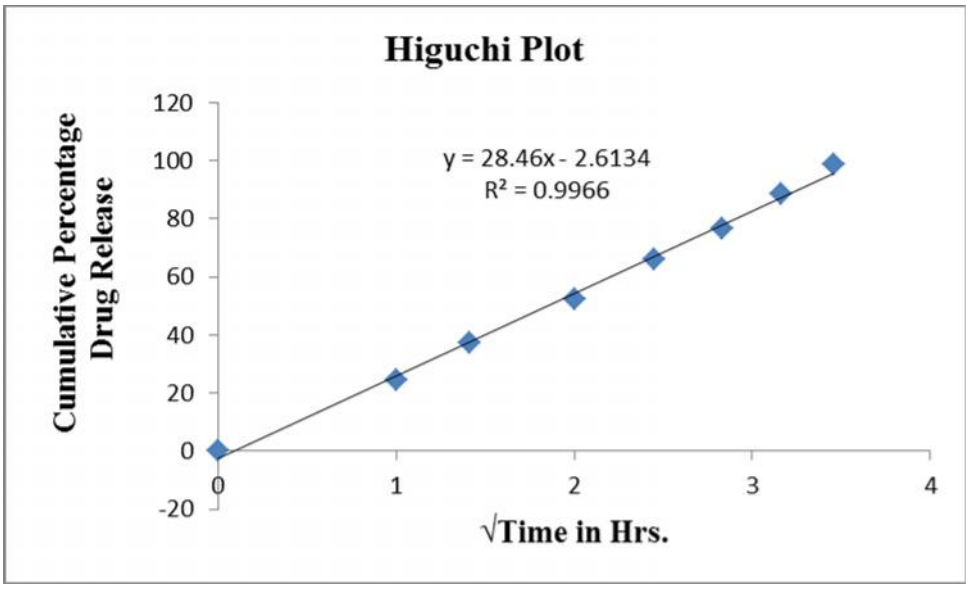


Figure No. 9.27: Higuchi Plot

Korsmeyer peppas plot

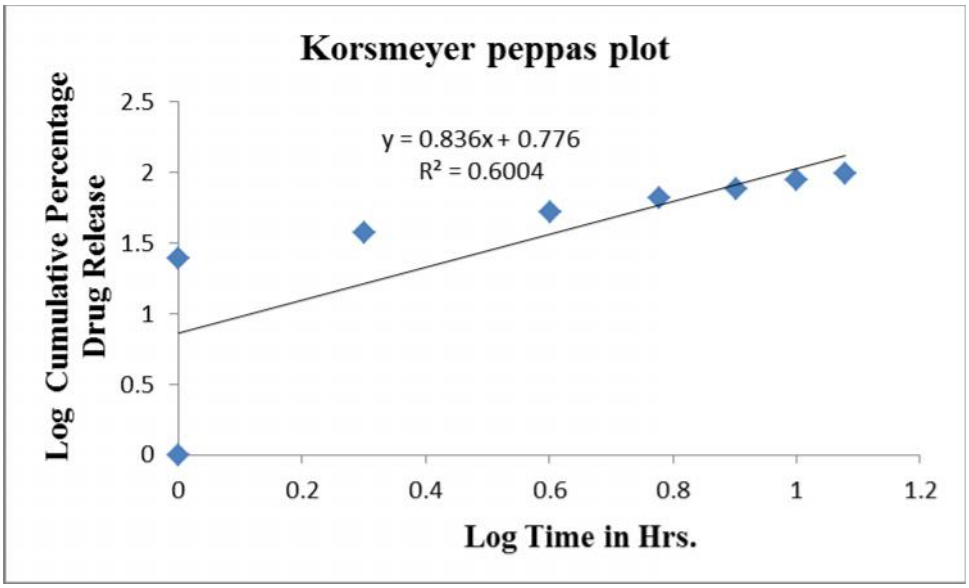


Figure No.9.28: Korsmeyer peppas plot

Table No.9.21: Kinetic values obtained from Zidovudine Layer (ZF5).

Formulation	Zero order plot R^2	First order plot R^2	Higuchi R^2	Korsmeyer-Peppas Plot R^2	N	Mechanism of Drug Release
ZF5	0.9862	0.8412	0.9966	0.6004	0.863	Zero order non fickian diffusion.

Mechanism of Drug Release

In order to understand the complex mechanism of drug releasevfrom the matrix system, the in vitro release rate were fitted to Korsmeyer-peppas model and interpretition of release component value (n) enlighten in understanding the release mechanism from the dosage form. The release exponent value (n) thus obtained was 0.863. The ZF5 formulation exhibited anomalous (non-fickian) diffusion mechanism.

These formulations are also showed as highest R^2 values of zero order kinetics indicating the amount of drug from the matrix system were by both diffusion and erosion.

STABILITY STUDIES (AS PER ICH GUIDELINES)

The fabricated controlled release formulation (finely selected LF5+ZF5) was subjected to stability studies at $40^{o}c\pm2^{o}c/75\%RH\pm5\%$ for 90 days. The product was evaluated for appearance and hardness for every 10 days. Drug content and drug release studies were conducted as per planned scheduled as above.

Table No.9.22: Showing drug content, hardness and friability study.

Duration	Drug content (%)		Hardness Kg/cm^2	Friability %
	Lamivudine	Zidovudine		
After one month	99.52	99.78	6.5±0.14	0.19
After two month	99.33	99.61	6.5±0.17	0.17
After three month	99.20	99.39	6.5±0.12	0.17

A] *In vitro* Dissolution studies For Lamivudine:

Table No.9.23:

Time in Hrs.	Cumulative Percentage Drug release		
	1st Month	2nd Month	3rd Month
0	0	0	0
1	20.27	20.01	19.76
2	32.28	32.10	31.88
4	48.71	48.45	48.14
6	62.50	62.23	61.97
8	74.41	74.20	73.90
10	85.54	85.38	85.13
12	97.71	97.53	97.25

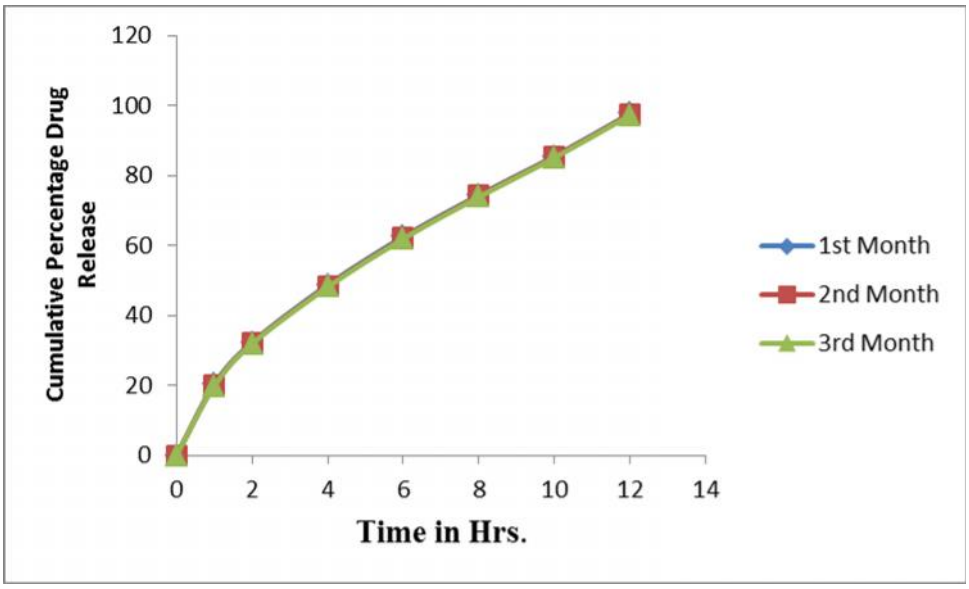


Fig No.9.29: Cumulative Percentage Drug release of formulation LF5 after 1^{st},2^{nd}& 3^{rd} month.

B] *In vitro* Dissolution studies For Zidovudine:

Table No.9.24:

Time in Hrs.	Cumulative Percentage Drug release		
	1^{st} Month	2^{nd} Month	3^{rd} Month
0	0	0	0
1	24.23	24.11	23.88
2	37.41	37.18	36.96
4	52.10	52.03	51.81
6	65.74	65.64	65.30
8	76.51	76.32	76.13
10	88.34	88.16	88.14
12	98.60	98.43	98.19

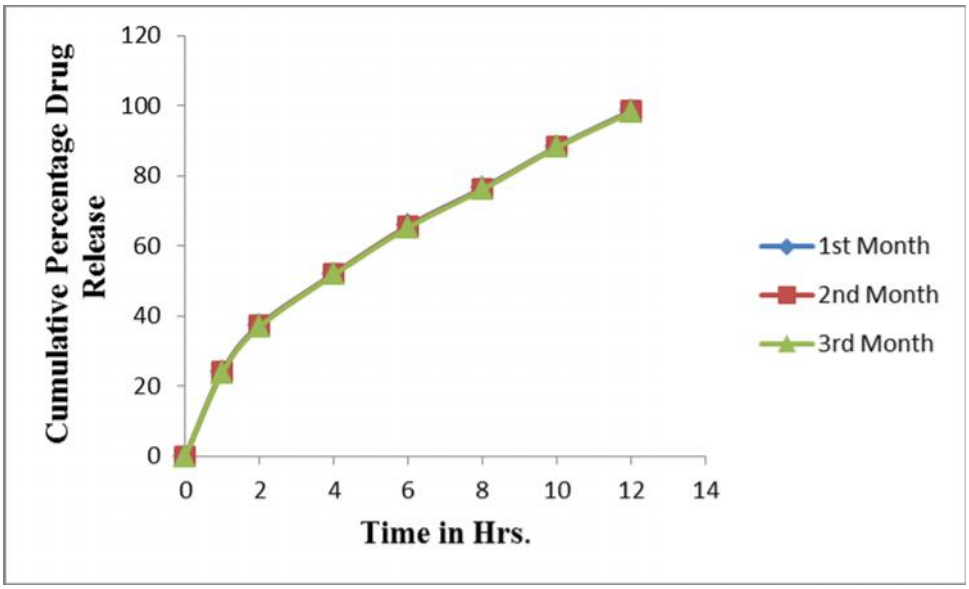


Figure No.9.30: Cumulative Percentage Drug release of formulation ZF5 after 1st, 2nd& 3rd month.

Chapter X

Summary

10. SUMMARY

Literature survey reveals that the various works has been done on the both synthetic and non-synthetic polymers. The successful preparations of the swellable matrices depend on the availability of proper selection of polymer. The present investigation was undertaken with the aim to formulate and *in vitro* evaluate Lamivudine and Zidovudine controlled release bilayer matrix tablets by using HPMC K 100 and HPMC K 15.

In the present work direct compression method was employed to prepare tablets. Micro-crystalline cellulose was selected as diluent. HPMC K 100 AND HPMC K 15 was used alone and combination as a rate controlling polymer in different concentration. Polyvinylpyrolidone was used as a binder. Magnesium stearate and aerosil were selected as lubricant and glidant respectively. Tablets were compressed individually using biconcave 8mm punches in Rimek multi station rotary compression machine.

Pre-compressional parameters like angle of repose, percentage compressibility and Hausner's ratio studies indicated that most of the formulation showed fair and good flow properties. These all pre-compression parameters were found within the limits. FTIR study was performed for the drug and excipient compatibility. From that study it showed there was no significant change in the peak value, which means there was no interaction between drug and excipients.

Post-compressional parameters like hardness, friability, drug content, dissolution studies were done for all formulation. All post-compressional parameters were found within the limits. From that studies I have selected best formulation and performed kinetic and stability studies.

The stability study for the best formulation (LF5+ZF5) were carried out by keeping the tablets at 45^0c± 2^0c/ 75% RH for 90 days. All the parameters were found to be within the limits after 90 days.

Investigation of the order and mechanism of drug release by plotting the in vitro data of best formulation (LF5+ZF5) for zero order and first order, Higuchi and

Korsmayer pappas equation were observed the slope value and regression co-efficient are given in table No. 9.19 and 9.21. The result of table showed that the formulation (LF5+ZF5) follow zero order and release the drug through polymeric membrane by following anomalous transport (Non-Fickian) diffusion.

From the study it can be concluded that the polymer viscosity, concentration as well as the nature of polymer play an important role in developing the controlled release dosage form.

Chapter XI

Conclusion

11. CONCLUSION

The study was undertaken with the aim to formulate the Lamivudine and Zidovudine controlled release bilayer matrix tablets by using different grades of polymer like HPMC (K100 and K 15) at different concentrations.

The polymer HPMC K 15 used in the formulation (LF5+ZF5) at 25%w/w of the drug showed the better controlled drug release (Lamivudine= 97.79% w/v and Zidovudine=98.68% w/v) upto 12 hrs. This was selected as the best formulation.

This formulation was reduce the frequent dosing, release the drug at controlled manner, increase efficacy, reduces the side effect and improved the patient's compliance. This combination reduces the risk of emergence of resistance to anti-retroviral drugs.

As it fulfills all the requirements of controlled release tablets and this study encourages further clinical trials and long term stability study for best formulation.

Chapter XII

Bibilography

11. BIBLIOGRAPHY

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